10/524,275

EAST Search History

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	19	AKAP84	US-PGPUB; USPAT; DERWENT	ADJ	ON	2007/02/13 12:09
L2	70229	fluorescen$ and (chimer$ or fusion)	US-PGPUB; USPAT; DERWENT	ADJ	ON	2007/02/13 12:10
L3	2107	fluorescen$.ab. and (chimer$ or fusion)	US-PGPUB; USPAT; DERWENT	ADJ	ON	2007/02/13 12:10
L4	902	fluorescen$.ab. and (chimer$ or fusion).ab.	US-PGPUB; USPAT; DERWENT	ADJ	ON	2007/02/13 12:11
L5	19	admin? and (chimer$ or fusion) and detect$	US-PGPUB; USPAT; DERWENT	ADJ	ON	2007/02/13 12:27
L6	119481	(chimer$ or fusion) and detect$	US-PGPUB; USPAT; DERWENT	ADJ	ON	2007/02/13 12:27
L7	16385	(chimer$ or fusion).ab. and detect$	US-PGPUB; USPAT; DERWENT	ADJ	ON	2007/02/13 12:27
L8	7531	(chimer$ or fusion).ab. and detect$.ab.	US-PGPUB; USPAT; DERWENT	ADJ	ON	2007/02/13 12:27
L9	157	X ray and l8	US-PGPUB; USPAT; DERWENT	ADJ	ON	2007/02/13 12:28
L10	218	resonance and l8	US-PGPUB; USPAT; DERWENT	ADJ	ON	2007/02/13 12:28
L11	172	l10 not l9	US-PGPUB; USPAT; DERWENT	ADJ	ON	2007/02/13 12:29
L12	329	l9 or l10	US-PGPUB; USPAT; DERWENT	ADJ	ON	2007/02/13 12:29

10/524275

1° = 8-20-02

File 5:Biosis Previews(R) 1969-2007/Feb W1
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*File 5: In preparation for coming enhancements, accession numbers will change soon. See HELP NEWS 5 for details.

Set	Items	Description
S1	532	((CHIMER? OR FUSION)(3W) PROTEIN) AND (RAY OR RESONANCE)
S2	53	S1 AND (BINDING()PROTEIN)
S3	0	((CHIMER? OR FUSION)(3W)PROTEIN(10W)FLUOROGEN?) AND RESONANCE
S4	0	((CHIMER? OR FUSION)(3W)PROTEIN(10W)CHROMOGEN?) AND RESONANCE
S5	1	((CHIMER? OR FUSION)(3W)PROTEIN(10W)CHROMOGEN?)
S6	669	REAGENT AND RESONANCE
S7	21	S6 AND ADMIN?
S8	12	S6 AND (FUSION OR CHIMER?)
S9	1283377	8 NOT S7
S10	11	S8 NOT S7
S11	3994	RESONANCE AND (FLUORESCEIN? OR CANINE? OR BIAMINE? OR XANTHINE OR RED OR BLUE OR RHODAMINE)
S12	0	S11 AND (FUSION OR CHIMER?) AND ADMIN?
S13	126	S11 AND (FUSION OR CHIMER?)
S14	0	S13 AND ADMINIST?
S15	200	S11 AND ADMINIST?
S16	0	S13 AND AKAP84
S17	14	AKAP84

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2/7/1
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0019442260 BIOSIS NO.: 200700102001
The T4 phage UvsW protein contains both DNA unwinding and strand annealing activities
AUTHOR: Nelson Scott W; Benkovic Stephen J (Reprint)
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JOURNAL: Journal of Biological Chemistry 282 (1): p407-416 JAN 5 2007 2007
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: UvsW protein belongs to the SF2 helicase family and is one of three helicases found in T4 phage. UvsW governs the transition from origin-dependent to origin-independent replication through the dissociation of R-loops located at the T4 origins of replication. Additionally, in vivo evidence indicates that UvsW plays a role in recombination-dependent replication and/or DNA repair. Here, the biochemical properties of UvsW helicase are described. UvsW is a 3' to 5' helicase that unwinds a wide variety of substrates, including those

resembling stalled replication forks and recombination intermediates. UvsW also contains a potent single-strand DNA annealing activity that is enhanced by ATP hydrolysis but does not require it. The annealing activity is inhibited by the non-hydrolysable ATP analog (adenosine 5'-O-(thiotriphosphate)), T4 single-stranded DNA-%%%binding%%% %%%protein%%% (gp32), or a small 8.8-kDa polypeptide (UvsW.1). Fluorescence %%%resonance%%% energy transfer experiments indicate that UvsW and UvsW. I form a complex, suggesting that the UvsW helicase may exist as a heterodimer in vivo. Fusion of UvsW and UvsW.1 results in a 68-kDa protein having nearly identical properties as the UvsW-UvsW.1 complex, indicating that the binding locus of UvsW.1 is close to the C terminus of UvsW. The biochemical properties of UvsW are similar to the RecQ protein family and suggest that the annealing activity of these helicases may also be modulated by protein-protein interactions. The dual activities of UvsW are well suited for the DNA repair pathways described for leading strand lesion bypass and synthesis-dependent strand annealing.

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19299820 BIOSIS NO.: 200600645215
Stabilization of a binary protein complex by intein-mediated cyclization
AUTHOR: Jeffries Cy M; Graham Stephen C; Stokes Philippa H; Collyer Charles A; Guss J Mitchell; Matthews Jacqueline M (Reprint)
AUTHOR ADDRESS: Univ Sydney, Sch Mol and Microbial Biosci, Sydney, NSW 2006, Australia**Australia
AUTHOR E-MAIL ADDRESS: j.matthews@mmb.usyd.edu.au
JOURNAL: Protein Science 15 (11): p2612-2618 NOV 2006 2006
ISSN: 0961-8368
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The study of protein-protein interactions can be hampered by the instability of one or more of the protein complex components. In this study, we showed that intein-mediated cyclization can be used to engineer an artificial intramolecular cyclic protein complex between two interacting proteins: the largely unstable LIM-only protein 4 (LMO4) and an unstructured domain of LIM domain %%%binding%%% %%%protein%%% 1 (ldb1). The X-%%%ray%%% structure of the cyclic complex is identical to noncyclized versions of the complex. Chemical and thermal denaturation assays using intrinsic tryptophan fluorescence and dynamic light scattering were used to compare the relative stabilities of the cyclized complex, the intermolecular (or free) complex, and two linear versions of the intramolecular complex (in which the interacting domains of LMO4 and ldb1 were fused, via a flexible linker, in either orientation). In terms of resistance to denaturation, the cyclic complex is the most stable variant and the intermolecular complex is the least stable; however, the two linear intramolecular variants show significant differences in stability. These differences appear to be related to the relative contact order (the average distance in sequence between residues that make contacts within a structure) of key binding residues at the interface of the two proteins. Thus, the restriction of the more stable component of a complex may enhance stability to a greater extent than restraining less

stable components.

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19199845 BIOSIS NO.: 200600545240
Refolding, purification and characterization of replication-initiator protein from soybean-infecting geminivirus
AUTHOR: Girish K R; Palanivelu S; Kumar P D; Usha R (Reprint)
AUTHOR ADDRESS: Madurai Kamaraj Univ, Sch Biotechnol, Dept Plant Biotechnol, Madurai 625021, Tamil Nadu, India**India
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JOURNAL: Journal of Virological Methods 136 (1-2): p154-159 SEP 2006 2006
ISSN: 0166-0934
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The replication-initiator protein (Rep) from a soybean-infecting geminivirus was overexpressed in E. coli as a %%%fusion%%% %%%protein%%% with maltose %%%binding%%% %%%protein%%% (MBP). In spite of the presence of the highly soluble MBP as the fusion partner, the overexpressed MBP-Rep %%%fusion%%% %%%protein%%% formed insoluble inclusion bodies. The protein was solubilized from the inclusion bodies and refolded. The refolded MBP-Rep protein was purified using ion exchange and amylose affinity chromatography. The activity of the purified MBP-Rep was assessed using an in vitro cleavage assay. Soluble and stable MBP-Rep protein was obtained in high abundance, providing the feasibility of large-scale production of active Rep protein for functional characterization and X-%%%ray%%% crystallographic structure determination. (c) 2006 Elsevier B.V. All rights reserved.

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19179549 BIOSIS NO.: 200600524944
The nuclear hormone receptor farnesoid X receptor (FXR) is activated by androsterone
AUTHOR: Wang Shuguang; Lai KehDih; Moy Franklin J; Bhat Anitha; Hartman Helen B; Evans Mark J (Reprint)
AUTHOR ADDRESS: Wyeth Res, Cardiovasc and Metab Dis Res, 500 Arcola Rd, Collegeville, PA 19426 USA**USA
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JOURNAL: Endocrinology 147 (9): p4025-4033 SEP 2006 2006
ISSN: 0013-7227
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Farnesoid X receptor (FXR) uses bile acids as endogenous ligands. Here, we demonstrate that androsterone, a metabolic product of testosterone, is also an FXR ligand. Treatment of castrated male mice with androsterone induced expression of the FXR target gene small

heterodimer partner (SHP). In mouse AML-12 hepatocytes, chenodeoxycholic acid (CDCA) or androsterone induced SHP expression with a similar kinetic pattern. The FXR antagonist guggulsterone blocked the induction of SHP by androsterone in AML-12 cells. Nuclear magnetic %%%resonance%%% spectroscopy demonstrated the direct binding of androsterone to purified human FXR (hFXR) ligand-binding domain (LBD) protein, resulting in the recruitment of steroid receptor coactivator protein-1 (SRC-1) coactivator peptide. In HEK293 cells, androsterone activated gal4-mouse FXR-LBD and gal4-hFXR-LBD fusion proteins, although in contrast to CDCA, androsterone activation was significantly greater for the mouse FXR-LBD than for the hFXR-LBD. Site-directed mutagenesis of the hFXR-LBD defined amino acids Asn354 and Ser345 as critical for differential species sensitivity to CDCA and androsterone, respectively. Crystal structure studies suggest that the orientation of the steroid nucleus of bile acids within the binding pocket of FXR is reversed from all other nuclear hormone receptors. In support of this model, we show here that mutations M265I or R331H, residues predicted by crystal structure to interact with the carboxylic acid tail of CDCA but not with androsterone, altered CDCA activation but had no effect on androsterone activation. Activation of FXR by androsterone may provide an additional means for physiological or pharmacological modulation of FXR.

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19074396 BIOSIS NO.: 200600419791
Activation of Rap1, Cdc42, and Rac by nectin adhesion system
BOOK TITLE: Methods in Enzymology
AUTHOR: Ogita Hisakazu (Reprint); Takai Yoshimi
BOOK AUTHOR/EDITOR: Balch WE (Editor); Der CJ (Editor); Hall A (Editor)
AUTHOR ADDRESS: Osaka Univ, Grad Sch Med, Dept Mol Biol and Biochem, Osaka, Japan**Japan
SERIES TITLE: METHODS IN ENZYMOLOGY 406 p415-424 2006
BOOK PUBLISHER: ELSEVIER ACADEMIC PRESS INC, 525 B STREET, SUITE 1900, SAN DIEGO, CA 92101-4495 USA
ISSN: 0076-6879 (print) ISBN: 0-12-182811-5 (H)
DOCUMENT TYPE: Book Chapter
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Nectin is an immunoglobulin-like cell-cell adhesion molecule and forms adherens junctions cooperatively with cadherin. Trans-interaction of nectin induces activation of Rap1, Cdc42, and Rac small G proteins. The activity of these small G proteins can be analyzed by the pull-down assay using GST-Ral-GDS %%%fusion%%% %%%protein%%% for Rap1 and GST-PAK-CRIB for Cdc42 and Rac. The fluorescent %%%resonance%%% energy transfer (FRET) system is also available to spatially and temporally detect the activity of these small G proteins in the living cells. In addition to these assays, the activity of Cdc42 and Rac is indirectly, but easily, evaluated by the cell-spreading assay to examine formation of filopodia and lamellipodia, respectively. To clearly explore the effect of trans-interacting nectin on the small G proteins, we use L fibroblasts stably expressing nectin-1 (nectin-1-L cells) and the extracellular domain of nectin-3 fused to human IgG Fc (Nef-3). Treatment of nectin-1-L cells with Nef-3 remarkably increases both the amount of the GTP-bound

form and the FRET efficiency of all Rap1, Cdc42, and Rac small G proteins. In the cell-spreading assay, Cdc42 and Rac activated in this way promote the formation of filopodia and lamellipodia, respectively. Here, we focus on how the activity of Cdc42 and Rac induced by trans-interacting nectin is examined by use of the pull-down and the cell-spreading assays.

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18808502 BIOSIS NO.: 200600153897
Probing the micro- and millisecond calcium dynamics of calmodulin.
AUTHOR: Allen Michael W (Reprint); Bieber Urbauer Ramona J; Johnson Carey K
AUTHOR ADDRESS: Univ Kansas, Dept Chem, Lawrence, KS 66045 USA**USA
AUTHOR E-MAIL ADDRESS: mallen@ku.edu
JOURNAL: Abstracts of Papers American Chemical Society 227 (Part 1): pU88 MAR 28 2004 2004
CONFERENCE/MEETING: 227th National Meeting of the American-Chemical Society Anaheim, CA, USA March 28 -April 01, 2004; 20040328
SPONSOR: Amer Chem Soc
ISSN: 0065-7727
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Citation
LANGUAGE: English

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18669092 BIOSIS NO.: 200600014487
Coupling of MBP %%%fusion%%% %%%protein%%% cleavage with sparse matrix crystallization screens to overcome problematic protein solubility
AUTHOR: Gruswitz Franz; Frishman Mary; Goldstein Barry M; Wedekind Joseph E (Reprint)
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AUTHOR E-MAIL ADDRESS: JosephWedekind@urmc.rochester.edu
JOURNAL: BioTechniques 39 (4): p476,478,480 OCT 2005 2005
ISSN: 0736-6205
DOCUMENT TYPE: Article
RECORD TYPE: Citation
LANGUAGE: English

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18367550 BIOSIS NO.: 200510062050
The acbH gene of Actinoplanes sp encodes a solute receptor with binding activities for acarbose and longer homologs
AUTHOR: Brunkhorst Claudia; Wehmeier Udo F; Piepersberg Wolfgang; Schneider Erwin (Reprint)
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JOURNAL: Research in Microbiology 156 (3): p322-327 APR 05 2005
ISSN: 0923-2508
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Acarbose, a pseudomaltotetraose, is produced by strains of the genus Actinoplanes and is a potent inhibitor of alpha-glucosidases, including those from the human intestine. Therefore, it is used in the treatment of patients suffering from type 2 diabetes. The benefits of acarbose for the producer are not known; however, besides acting as an inhibitor of alpha-amylases secreted by competitors, a role as a 'carbophor' has been proposed. This would require a transport system mediating its uptake into the cytoplasm of Actinoplanes sp. A putative sugar ATP binding cassette (ABC) transport system, the genes of which are included within the biosynthetic gene cluster for acarbose, was suggested to be a possible candidate. The genes acbHFG encode a possible sugar %%%binding%%% %%%protein%%% (AcbH) and two membrane integral subunits (AcbFG). A gene coding for an ATPase component is missing. Since Actinoplanes sp. cannot yet be genetically manipulated we performed experiments to identify the substrate(s) of the putative transporter by assessing the substrate specificity of AcbH. The protein was overproduced in Escherichia coli as His(10)-%%%fusion%%% %%%protein%%%, purified under denaturating conditions and renatured. Refolding was verified by circular dichroism spectroscopy. Surface plasmon %%%resonance%%% studies revealed that AcbH binds acarbose and longer derivatives, but not maltodextrins, maltose or sucrose. Immunoblot analysis revealed the association of AcbH with the membrane fraction of Actinoplanes cells that were grown in the presence of maltose, maltodextrins or acarbose. Together, these findings suggest that the AcbHFG complex might be involved in the uptake of acarbose and are consistent with a role for acarbose as a 'carbophor'.
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18313666 BIOSIS NO.: 200510008166
Rapid, high-yield expression and purification of Ca2+-ATPase regulatory proteins for high-resolution structural studies
AUTHOR: Douglas Jennifer L; Trieber Catharine A; Afara Michael; Young Howard S (Reprint)
AUTHOR ADDRESS: Univ Alberta, Dept Biochem, Edmonton, AB T6G 2H7, Canada** Canada
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JOURNAL: Protein Expression and Purification 40 (1): p118-125 MAR 05 2005
ISSN: 1046-5928
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Phospholamban (PLB) and sarcolipin (SLN) are small integral membrane proteins that regulate the Ca2+-ATPases of cardiac and skeletal muscle, respectively, and directly alter their calcium transport

properties. PLB interacts with and regulates the cardiac Ca2+-ATPase at submaximal calcium concentrations, thereby slowing relaxation rates and reducing contractility in the heart. SLN interacts with and regulates the skeletal muscle Ca2+-ATPase in a mechanism analogous to that used by PLB. While these regulatory interactions are biochemically and physiologically well characterized, structural details are lacking. To pursue structural studies, such as electron cryo-microscopy and X-%%%ray%%% crystallography, large quantities of over-expressed and purified protein are required. Herein, we report a modified method for producing large quantities of PLB and SLN in a rapid and efficient manner. Briefly, recombinant wild-type PLB and SLN were over-produced in Escherichia coli as maltose %%%binding%%% %%%protein%%% fusion proteins. A tobacco etch virus protease site allowed specific cleavage of the %%%fusion%%% %%%protein%%% and release of recombinant PLB or SLN. Selective solubilization with guanidine-hydrochloride followed by reverse-phase HPLC permitted the rapid, large-scale production of highly pure protein. Reconstitution and measurement of ATPase activity confirmed the functional interaction between our recombinant regulatory proteins and Ca2+-ATPase. The inhibitory properties of the over-produced proteins were consistent with previous studies, where the inhibition was relieved by elevated calcium concentrations. In addition, we show that our recombinant PLB and SLN are suitable for high-resolution structural studies. (c) 2004 Elsevier Inc. All rights reserved.

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18269996 BIOSIS NO.: 200500176732
Development and use of fluorescent nanosensors for metabolite imaging in living cells
AUTHOR: Fehr M; Okumoto S; Deuschle K; Lager I; Looger L L; Persson J; Kozhukh L; Lalonde S; Frommer W B (Reprint)
AUTHOR ADDRESS: Carnegie Inst, 260 Panama St, Stanford, CA, 94305, USA**USA
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JOURNAL: Biochemical Society Transactions 33 (Part 1): p287-290 February 2005 2005
MEDIUM: print
ISSN: 0300-5127
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: To understand metabolic networks, fluxes and regulation, it is crucial to be able to determine the cellular and subcellular levels of metabolites. Methods such as PET and NMR imaging have provided us with the possibility of studying metabolic processes in living organisms. However, at present these technologies do not permit measuring at the subcellular level. The comeleon, a fluorescence %%%resonance%%% energy transfer (FRET)-based nanosensor uses the ability of the calcium-bound form of calmodulin to interact with calmodulin binding polypeptides to turn the corresponding dramatic conformational change into a change in %%%resonance%%% energy transfer between two fluorescent proteins attached to the %%%fusion%%% %%%protein%%%. The cameleon and its derivatives were successfully used to follow calcium changes in real time not only in isolated cells, but also in living organisms. To provide a set of tools

for real-time measurements of metabolite levels with subcellular resolution, protein-based nanosensors for various metabolites were developed. The metabolite nanosensors consist of two variants of the green fluorescent protein fused to bacterial periplasmic binding proteins. Different from the cameleon, a conformational change in the %%%binding%%% %%%protein%%% is directly detected as a change in FRET efficiency. The prototypes are able to detect various carbohydrates such as ribose, glucose and maltose as purified proteins in vitro. The nanosensors can be expressed in yeast and in mammalian cell cultures and were used to determine carbohydrate homeostasis in living cells with subcellular resolution. one future goal is to expand the set of sensors to cover a wider spectrum of metabolites by using the natural spectrum of bacterial periplasmic binding proteins and by computational design of the binding pockets of the prototype sensors.

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17978051 BIOSIS NO.: 200400348840
A %%%fusion%%% %%%protein%%% expression analysis using surface plasmon %%%resonance%%% imaging
AUTHOR: Jung Jin-Mi; Shin Yong-Beom; Kim Min-Gon; Ro Hyeon-Su; Jung Hee-Tae ; Chung Bong Hyun (Reprint)
AUTHOR ADDRESS: BioNanotechnol Res Ctr, Korea Res Inst Biosci and Biotechnol, POB 115, Taejon, 305600, South Korea**South Korea
AUTHOR E-MAIL ADDRESS: chungbh@kribb.re.kr
JOURNAL: Analytical Biochemistry 330 (2): p251-256 July 15, 2004 2004
MEDIUM: print
ISSN: 0003-2697 _(ISSN print)
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: A surface plasmon %%%resonance%%% (SPR) imaging system was constructed and used to detect the affinity-tagged recombinant proteins expressed in Escherichia coli. With regards to model proteins, the hexahistidine-ubiquitin-tagged human growth hormone (His6-Ub-hGH), glutathione S-transferase-tagged human interleukin-6 (GST-hIL6), and maltose-%%%binding%%% %%%protein%%%-tagged human interleukin-6 (MBP-hIL6) expressed in E. coli were analyzed. The cell lysates were spotted on gold thin films coated with 11-mercaptoundecanol (MUOH)/dextran derivatized with Ni(II)-iminodiacetic acid (IDA-Ni(II)), glutathione, or cyclodextrin. After a brief washing of the gold chip, SPR imaging measurements were carried out in order to detect the bound affinity-tagged fusion proteins. Using this new approach, rapid high-throughput expression analysis of the affinity-tagged proteins were obtained. The SPR imaging protein chip system used to measure the expression of affinity-tagged proteins in a high-throughput manner is expected to be an attractive alternative to traditional laborious and time-consuming methods, such as SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blots. Copyright 2004 Elsevier Inc. All rights reserved.

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17855426 BIOSIS NO.: 200400225481
Expression of the soluble extracellular domain of human thrombopoietin receptor using a maltose-%%%binding%%% %%%protein%%%-affinity fusion system.
AUTHOR: Zhang Qing; Pan Rui-Min; Ge Yi-Chen; Xu Peilin (Reprint)
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AUTHOR E-MAIL ADDRESS: xupeilin@hotmail.com
JOURNAL: Biological & Pharmaceutical Bulletin 27 (2): p219-221 February 2004 2004
MEDIUM: print
ISSN: 0918-6158
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The thrombopoietin (TPO) receptor (Mpl) belongs to the family of ligand-dependent cytokine receptors and plays a functional role in regulating platelet production. The signaling capacity largely depends on the binding of TPO to the extracellular domains of the TPO receptor (Mpl-EC). Because the expression level of Mpl in human tissue is very low, studies on the functional and spatial characteristics of its ligand-binding sites have been limited. In the present study, we report the expression and purification of Mpl-EC as a fusion with the maltose-%%%binding%%% %%%protein%%% (MBP), designated MBP-Mpl-EC. MBP-Mpl-EC was expressed in the cytoplasm of Escherichia coli as a soluble %%%fusion%%% %%%protein%%%. Specific binding of TPO to purified MBP-Mpl-EC was demonstrated by a dot-blot assay and surface plasmon %%%resonance%%%. We conclude that bacterial expression of MBP-Mpl-EC yields large amounts of protein with correct folding and that it can be used for further structure and function analyses.

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17807396 BIOSIS NO.: 200400178153
FRET evidence for a conformational change in TFIIB upon TBP-DNA binding.
AUTHOR: Zheng Le; Hoeflich Klaus P; Elsby Laura M; Ghosh Mahua; Roberts Stefan G E; Ikura Mitsuhiko (Reprint)
AUTHOR ADDRESS: Division of Molecular and Structural Biology, Department of Medical Biophysics, Ontario Cancer Institute, University of Toronto, 610 University Avenue, Toronto, ON, M5G 2M9, Canada**Canada
AUTHOR E-MAIL ADDRESS: mikura@uhnres.utoronto.ca
JOURNAL: European Journal of Biochemistry 271 (4): p792-800 February 2004 2004
MEDIUM: print
ISSN: 0014-2956 _(ISSN print)
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: As a critical step of the preinitiation complex assembly in transcription, the general transcription factor TFIIB forms a complex with the TATA-box %%%binding%%% %%%protein%%% (TBP) bound to a promoter element. Transcriptional activators such as the herpes simplex virus VP16 facilitate this complex formation through conformational activation of TFIIB, a focal molecule of transcriptional initiation and activation. Here, we used fluorescence %%%resonance%%% energy transfer to investigate conformational states of human TFIIB fused to enhanced cyan fluorescent protein and enhanced yellow fluorescent protein at its N- and C-terminus, respectively. A significant reduction in fluorescence %%%resonance%%% energy transfer ratio was observed when this %%%fusion%%% %%%protein%%%, hereafter named CYIIB, was mixed with promoter-loaded TBP. The rate for the TFIIB-TBP-DNA complex formation is accelerated drastically by GAL4-VP16 and is also dependent on the type of promoter sequences. These results provide compelling evidence for a 'closed-to-open' conformational change of TFIIB upon binding to the TBP-DNA complex, which probably involves alternation of the spatial orientation between the N-terminal zinc ribbon domain and the C-terminal conserved core domain responsible for direct interactions with TBP and a DNA element.

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17801864 BIOSIS NO.: 200400172621
Structural and functional basis of the dominant negative phenotype of AML1-ETO, product of the t(8;21).
AUTHOR: Bushweller John H (Reprint); Liu Yizhou (Reprint); Chruszcz Maksymilian (Reprint); Minor Wladek (Reprint); Speck Nancy A
AUTHOR ADDRESS: Molecular Physiology and Biological Physics, University of Virginia, Charlottesville, VA, USA**USA
JOURNAL: Blood 102 (11): p171a November 16, 2003 2003
MEDIUM: print
CONFERENCE/MEETING: 45th Annual Meeting of the American Society of Hematology San Diego, CA, USA December 06-09, 2003; 20031206
SPONSOR: American Society of Hematology
ISSN: 0006-4971
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Chromosomal rearrangements that target the core-binding factor genes are some of the most common mutations in leukemia. RUNX1 (AML1), which encodes a DNA-binding subunit of a core-binding factor (CBF) is disrupted by numerous translocations, all of which are associated with myeloid and lymphocytic leukemia. In all cases, a %%%fusion%%% %%%protein%%% that contains the DNA- and CBFbeta-binding Runt domain of Runx1 is generated as a result of the translocation. Thus, the Runx1 fusion proteins are capable of binding Runx1-CBFbeta target sites in the genome and of potentially dysregulating the expression of multiple genes required for normal hematopoiesis. The t(8;21) associated with AML (M2 subtype) fuses together the N-terminal 177 amino acids of RUNX1 to virtually all of ETO. Knock-in of the AML1-ETO gene into the Runx1 locus in mice causes embryonic lethality with the same phenotype as that observed for knockout of either the Runx1 or Cbfb genes, namely extensive hemorrhaging and a profound blockage in hematopoietic development. Thus,

AML1-ETO acts as a dominant inhibitor of Runx1-CBF function. In addition, a conditional knock-in model has been used to show that AML1-ETO highly predisposes mice to the development of leukemia. All of these results are consistent with AML1-ETO playing a critical role in leukemogenesis. The ETO protein, also known as MTG8 (myeloid tumor gene 8), is the human homolog of the Drosophila Nervy protein, and shares 4 homologous domains termed NHR1-4. The second Nervy homology domain (NHR2) is referred to as the HHR domain. This domain has been shown to mediate oligomerization among ETO family members. Deletion of the HHR (NHR2) domain resulted in loss of transcriptional repression, and abolished the block in terminal differentiation of human promonocytic U937 cells induced by AML1-ETO. We have recently solved the three-dimensional structure of the HHR domain (NHR2) by x-%%%ray%%% crystallography. Both the structure and sedimentation velocity measurements show that the HHR domain is a tetramer. The structure forms a 4-helix bundle arrangement, however this bundle appears to be unique as a search of the SCOP database turned up only 1 structural homolog. As a result of the tetrameric nature of the HHR domain of ETO, fusion of RUNX1 to ETO makes AML1-ETO a poly-valent DNA-%%%binding%%% %%%protein%%% in contrast to the mono-valent RUNX1. It is our hypothesis that one of the reasons AML1-ETO dominantly inhibits Runx1-CBFbeta function is that it binds to DNA more tightly than does RUNX1 itself, thereby competing RUNX1 off the DNA. This concept derives from the known thermodynamics of poly-valent versus mono-valent protein interactions with ligands containing multiple binding sites. The creation of a poly-valent %%%binding%%% %%%protein%%% will in almost all cases result in higher affinity binding than for a mono-valent version of the same protein because the Kd is a product of the individual binding constants. Many of the sites to which RUNX1 binds contain multiple RUNX1 binding sites in close proximity to one another, thereby providing exactly the conditions necessary to have higher affinity binding of AML1-ETO. We have made measurements of the binding of a functional fragment of AML1-ETO to a TCRbeta enhancer element containing two RUNX binding sites and shown that indeed AML1-ETO binds with higher affinity to this DNA element than does RUNX1. We believe this effect is critical for the dominant negative phenotype of AML1-ETO and for the associated leukemogenesis. In-vivo testing of this effect is underway.

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17776113 BIOSIS NO.: 200400156870
Fluorescence labeling, purification, and immobilization of a double cysteine mutant calmodulin %%%fusion%%% %%%protein%%% for single-molecule experiments.
AUTHOR: Allen Michael W; Urbauer Ramona J Bieber; Zaidi Asma; Williams Todd D; Urbauer Jeffrey L; Johnson Carey K (Reprint)
AUTHOR ADDRESS: Department of Chemistry, University of Kansas, Lawrence, KS, 66045, USA**USA
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JOURNAL: Analytical Biochemistry 325 (2): p273-284 February 15, 2004 2004
MEDIUM: print
ISSN: 0003-2697 _(ISSN print)
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: We present a method of labeling and immobilizing a low-molecular-weight protein, calmodulin (CaM), by %%%fusion%%% to a larger %%%protein%%%, maltose %%%binding%%% %%%protein%%% (MBP), for single-molecule fluorescence experiments. Immobilization in an agarose gel matrix eliminates potential interactions of the protein and the fluorophore(s) with a glass surface and allows prolonged monitoring of protein dynamics. The small size of CaM hinders its immobilization in low-weight-percentage agarose gels; however, fusion of CaM to MBP via a flexible linker provides sufficient restriction of translational mobility in 1% agarose gels. Cysteine residues were engineered into MBPcntdotCaM (MBP-T34C,T110C-CaM) and labeled with donor and acceptor fluorescent probes yielding a construct (MBPcntdotCaM-DA) which can be used for single-molecule single-pair fluorescence %%%resonance%%% energy transfer (spFRET) experiments. Mass spectrometry was used to verify the mass of MBPcntdotCaM-DA. Assays measuring the activity of CaM reveal minimal activity differences between wild-type CaM and MBPcntdotCaM-DA. Single-molecule fluorescence images of the donor and acceptor dyes were fit to a two-dimensional Gaussian function to demonstrate colocalization of donor and acceptor dyes. FRET is demonstrated both in bulk fluorescence spectra and in fluorescence trajectories of single MBPcntdotCaM-DA molecules. The extension of this method to other biomolecules is also proposed.

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17769898 BIOSIS NO.: 200400137252
Three-dimensional position and orientation of synaptotagmin I C2AB on PS/PC membranes using site directed spin labeling.
AUTHOR: Sterbling Stephenie (Reprint); Frazier April (Reprint); Roller Christina (Reprint); Cafiso David (Reprint)
AUTHOR ADDRESS: Chemistry, University of Virginia, Charlottesville, VA, USA **USA
JOURNAL: Biophysical Journal 86 (1): p558a January 2004 2004
MEDIUM: print
CONFERENCE/MEETING: 48th Annual Meeting of the Biophysical Society Baltimore, MD, USA February 14-18, 2004; 20040214
SPONSOR: Biophysical Society
ISSN: 0006-3495 _(ISSN print)
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: C2 domains function in Ca2+-dependent membrane binding and contain rigid eight-stranded beta-sandwiches with flexible loops connecting the strands. On one end of the domain, these loops coordinate Ca2+ ions. Synaptotagmin I (syn I) is a calcium dependent membrane %%%binding%%% %%%protein%%%, which is hypothesized to be the synaptic %%%fusion%%% triggering %%%protein%%%. Syn I consists of two similar C2 domains called C2A and C2B, which are connected by a flexible linker. Syn I is attached to the synaptic vesicle via a transmembrane domain linked to C2A. Ca2+ binding appears to alter the electrostatics and/or hydration energy of the loop regions making membrane attachment favorable. Previous work using site-directed spin labeling (SDSL) indicates that the

backbones of the calcium binding loops 1 and 3 of syn I C2A lie at or on the hydrocarbon side of the lipid phosphates in negatively charged phosphatidycholine (PC):phosphatidylserine (PS) membranes. The backbone of loop 3 penetrates approximately 5ANG below the level of the lipid phosphates. There is evidence that the interactions of C2A with membranes may be modified by the presence of C2B. Using SDSL along with electron paramagnetic %%%resonance%%% spectroscopy (EPR), we investigated the orientation and position of syn I C2AB on PC:PS bilayers. The EPR spectra obtained for C2A are similar to those generated from the C2AB construct. In addition, the average depth of penetration of C2A does not appear to be altered significantly by the presence of C2B. Models comparing the orientation and position syn I C2A and C2AB on the lipid bilayer will be presented.

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17742206 BIOSIS NO.: 200400111912
Photoreceptor cGMP phosphodiesterase delta subunit (PDEdelta) functions as a prenyl-%%%binding%%% %%%protein%%%.
AUTHOR: Zhang Houbin; Liu Xiao-hui; Zhang Kai; Chen Ching-Kang; Frederick Jeanne M; Prestwich Glenn D; Baehr Wolfgang (Reprint)
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JOURNAL: Journal of Biological Chemistry 279 (1): p407-413 January 2, 2004 2004
MEDIUM: print
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Bovine PDEdelta was originally copurified with rod cGMP phosphodiesterase (PDE) and shown to interact with prenylated, carboxymethylated C-terminal Cys residues. Other studies showed that PDEdelta can interact with several small GTPases including Rab13, Ras, Rap, and Rho6, all of which are prenylated, as well as the N-terminal portion of retinitis pigmentosa GTPase regulator and Arl2/Arl3, which are not prenylated. We show by immunocytochemistry with a PDEdelta-specific antibody that PDEdelta is present in rods and cones. We find by yeast two-hybrid screening with a PDEdelta bait that it can interact with farnesylated rhodopsin kinase (GRK1) and that prenylation is essential for this interaction. In vitro binding assays indicate that both recombinant farnesylated GRK1 and geranylgeranylated GRK7 co-precipitate with a glutathione S-transferase-PDEdelta %%%fusion%%% %%%protein%%%. Using fluorescence %%%resonance%%% energy transfer techniques exploiting the intrinsic tryptophan fluorescence of PDEdelta and dansylated prenyl cysteines as fluorescent ligands, we show that PDEdelta specifically binds geranylgeranyl and farnesyl moieties with a Kd of 19.06 and 0.70 gammaM, respectively. Our experiments establish that PDEdelta functions as a prenyl-%%%binding%%% %%%protein%%% interacting with multiple prenylated proteins.

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17612990 BIOSIS NO.: 200300581709
Preliminary structure analysis of the DH/PH domains of leukemia-associated RhoGEF.
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JOURNAL: Acta Crystallographica Section D Biological Crystallography 59 (10): p1859-1862 October 2003 2003
MEDIUM: print
ISSN: 0907-4449
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RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Leukemia-associated RhoGEF (LARG) is a multidomain protein that relays signals from Galpha12/13-coupled heptahelical receptors to GTPases that regulate the cytoskeleton. To understand the molecular basis of LARG-mediated signal transduction, structural analysis of its DH/PH domains has been initiated. The LARG DH/PH domains have been overexpressed in Escherichia coli as a TEV protease-cleavable %%%fusion%%% %%%protein%%% containing maltose-%%%binding%%% %%%protein%%% and a hexahistidine tag at the N- and C-termini, respectively. Crystals of the DH/PH domains were obtained (space group C2; unit-cell parameters a=195.5, b=46.0, c=75.1 ANG, beta=105.0degree) and xenon and NaBr derivatives were generated which should allow the structure to be determined by MIRAS.

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17557334 BIOSIS NO.: 200300512697
THE PUTATIVE CGMP PHOSPHODIESERASE delta SUBUNIT ("PDEdelta") FUNCTIONS AS A PRENYL %%%BINDING%%% %%%PROTEIN%%% (PBP)
AUTHOR: Zhang H (Reprint); Liu X -H; Zhang K; Chen C -K; Frederick J M; Prestwich G D; Baehr W
AUTHOR ADDRESS: Ophthalmology, Neurobiology and Anatomy, Neurobiology and AMPAnatomy, Biology, University of Utah, Salt Lake City, UT, USA**USA
JOURNAL: ARVO Annual Meeting Abstract Search and Program Planner 2003 p Abstract No. 1523 2003 2003
MEDIUM: cd-rom
CONFERENCE/MEETING: Annual Meeting of the Association for Research in Vision and Ophthalmology Fort Lauderdale, FL, USA May 04-08, 2003; 20030504
SPONSOR: Association for Research in Vision and Ophthalmology
DOCUMENT TYPE: Meeting; Meeting Poster; Meeting Abstract
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Purpose: To define the role and function of "PDEdelta", a

putative subunit of rod cGMP phosphodiesterase (PDE). Methods: Immunocytochemistry with bovine "PDEdelta" specific polyclonal antibody. Yeast 2-hybrid (y2h) screening of a bovine retina cDNA expression library with a "PDEdelta" bait construct cloned into pGBKT7. GST pull down assays to verify stable binding and interactions of y2h positive clones with "PDEdelta". Fluorescence %%%resonance%%% energy transfer (FRET) with dansylated prenyl ligands to determine dissociation constants. Results: "PDEdelta" was originally shown to copurify with rod PDE from bovine retina and thought to be a fourth subunit of PDE. However, other studies showed that "PDEdelta" can interact with several small GTPases, including Rab13, Ras, Rap, and the retinitis pigmentosa GTPase regulator (RPGR), all of which are prenylated, as well as Arl2/Arl3 which are not known to be modified. Immunocytochemistry with a "PDEdelta" specific antibody shows distribution of "PDEdelta" strongly in cones, weaker in rods and other cell types of the bovine retina. We find by y2h screening with a "PDEdelta" bait that it can interact with rhodopsin kinase (GRK1), and that prenylation (GRK1 is farnesylated) is essential for this interaction. When the C-terminal CAAX box motif signaling prenylation is mutated (C to S), the mutant GRK1 does not associate with "PDEdelta". In-vitro binding assays (GST pull downs) indicate that both recombinant GRK1 and GRK7 (which is geranylgeranylated) co-precipitate with a GST-"PDEdelta" %%%fusion%%% %%%protein%%%. Using fluorescence %%%resonance%%% energy transfer techniques exploiting the intrinsic tryptophan fluorescence of "PDEdelta" and dansylated prenyl chains as fluorescent ligands, we show that "PDEdelta" specifically binds geranylgeranyl and farnesyl moieties with a Kd of 19.0 muM and 0.70 muM, respectively. Conclusions: "PDEdelta" can be detected at high levels in bovine cones, thus it is not an exclusive subunit of rod PDE. Our experiments establish that "PDEdelta" can function as a prenyl %%%binding%%% %%%protein%%% (PBP) interacting with multiple prenylated proteins. Its precise function when interacting with hydrophobic tails in a GDI-like fashion is unknown. PDEdelta's function is not limited to prenyl binding since it also interacts with non-prenylated proteins. We propose to rename "PDEdelta" into prenyl %%%binding%%% %%%protein%%% (PBP).

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17530690 BIOSIS NO.: 200300488347
Purification, crystallization and preliminary X-%%%ray%%% analysis of a Sco1-like protein from Bacillus subtilis, a copper-%%%binding%%% %%%protein%%% involved in the assembly of cytochrome c oxidase.
AUTHOR: Imriskova-Sosova Iveta; Ye Qilu; Hill Bruce C (Reprint); Jia Zongchao
AUTHOR ADDRESS: Department of Biochemistry, Queen's University, Kingston, ON, K7L 3N4, Canada**Canada
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JOURNAL: Acta Crystallographica Section D Biological Crystallography 59 (7): p1299-1301 July 2003 2003
MEDIUM: print
ISSN: 0907-4449
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The putative copper-delivery protein BsSco from Bacillus subtilis is a member of the Sco family of cytochrome c oxidase assembly proteins. BsSco is a membrane protein and the soluble domain has been cloned and expressed in Escherichia coli as a fusion with glutathione-S-transferase. The %%%fusion%%% %%%protein%%% was isolated from the cell lysate using a glutathione-affinity column and the soluble domain of BsSco was released by treatment with thrombin. Sufficient amounts of the soluble domain have been obtained for crystallization. Crystals obtained by hanging-drop vapour diffusion diffract to a resolution of 2.3 ANG at a synchrotron source. The space group is P6 and the unit-cell parameters are a=67.74, b=67.74, c=189.58 ANG.

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17485187 BIOSIS NO.: 200300442221
Flow cytometric measurement of fluorescence (Forster) %%%resonance%%% energy transfer from cyan fluorescent protein to yellow fluorescent protein using single-laser excitation at 458 nm.
AUTHOR: He Liusheng; Bradrick Thomas D; Karpova Tatiana S; Wu Xiaoli; Fox Michael H; Fischer Randy; McNally James G; Knutson Jay R; Grammer Amrie C ; Lipsky Peter E (Reprint)
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JOURNAL: Cytometry 53A (1): p39-54 May 2003 2003
MEDIUM: print
ISSN: 0196-4763 _(ISSN print)
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Background: Use of distinct green fluorescent protein (GFP) variants permits the study of protein-protein interactions and colocalization in viable transfected cells by fluorescence (Forster) %%%resonance%%% energy transfer (FRET). Flow cytometry is a sensitive method to detect FRET. However, the typical dual-laser methods used in flow cytometric FRET assays are not generally applicable because they require a specialized krypton ultraviolet (UV) laser. The purpose of this work was to develop a flow cytometric method to detect FRET between cyan fluorescent protein (CFP; donor) and yellow fluorescent protein (YFP; acceptor) by using the 458-nm excitation from a single tunable argon-ion laser. Methods: FUSE-%%%binding%%% %%%protein%%% (FBP) interacting repressor (FIR) and FBP are c-myc transcription factors and are known to interact physically. To examine their interaction within viable cells, FIR and the binding motif of FBP, the FBP central domain (FBPcd), were fused with CFP and YFP, respectively, and this pair of fluorescently-tagged proteins was used to detect FRET in vivo. Cells transfected with expression plasmids encoding a CFP-FIR %%%fusion%%% %%%protein%%% and YFP as a negative control, a CFP-YFP %%%fusion%%% %%%protein%%% as a positive control, or CFP-FIR and YFP-FBPcd fusion proteins were examined for FRET after excitation with a 458-nm line from a tunable argon-ion laser. FRET was measured as the ratio of YFP:CFP emission or as YFP emission at 564-606 nm. Conventional FRET using the

413-nm UV line from a krypton laser was examined for comparison. Fluorescence signals were separated with a customized optical filter configuration using 530-nm shortpass, 500-nm longpass, and 560-nm shortpass dichroics in addition to 488/30 nm (CFP), 530/30 nm (YFP), and 585/42 nm (FRET) bandpass filters. Further, a laser-scanning confocal microscopic photobleach technique was used to document that FRET occurred by showing that the intensity of donor CFP fluorescence increased after its acceptor YFP was photobleached. Steady-state spectrofluorometry was used to confirm and validate the results detected by flow cytometry. Results: Upon excitation with the 458-nm line of the argon-ion laser, the enhancement of the acceptor YFP signal and the decrease of the CFP signal were easily detected in cells transfected with the CFP-YFP construct or CFP-FIR and YFP-FBPcd. Similarly, FRET was detected under these conditions when the YFP emission was assessed at 564-606 nm. A strong correlation was observed between the increase in the YFP:CFP ratio and the YFP emission detected at 564-606 nm, consistent with the conclusion that FRET was detected comparably by both methods. A conventional flow cytometric krypton UV-laser technique was also used to confirm that FRET occurred with the CFP-YFP %%%fusion%%% %%%protein%%% and from CFP-FIR fwdarw YFP-FBPcd. FRET also was confirmed by a confocal photo-bleaching technique, in which donor CFP intensity was enhanced after its acceptor YFP was photobleached. The flow cytometric and confocal microscopic results were confirmed by spectrofluorometry. Conclusion: These results demonstrated the feasibility of flow cytometric detection of FRET signals from CFP to YFP by excitation with the 458-nm line from the tunable argon-ion laser. The method was as efficient as excitation with the krypton UV laser and therefore should make FRET a more generally available flow cytometric technique.

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17481300 BIOSIS NO.: 200300438334
Crystal structures of fusion proteins with large-affinity tags.
AUTHOR: Smyth Douglas R; Mrozkiewicz Marek K; McGrath William J; Listwan Pawel; Kobe Bostjan (Reprint)
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JOURNAL: Protein Science 12 (7): p1313-1322 July 2003 2003
MEDIUM: print
ISSN: 0961-8368
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RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The %%%fusion%%% of a %%%protein%%% of interest to a large-affinity tag, such as the maltose-%%%binding%%% %%%protein%%% (MBP), thioredoxin (TRX), or glutathione-S-transferase (GST), can be advantageous in terms of increased expression, enhanced solubility, protection from proteolysis, improved folding, and protein purification via affinity chromatography. Unfortunately, crystal growth is hindered by the conformational heterogeneity induced by the fusion tag, requiring that the tag is removed by a potentially problematic cleavage step. The first three crystal structures of fusion proteins with large-affinity

tags have been reported recently. All three structures used a novel strategy to rigidly fuse the protein of interest to MBP via a short three- to five-amino acid spacer. This strategy has the potential to aid structure determination of proteins that present particular experimental challenges and are not conducive to more conventional crystallization strategies (e.g., membrane proteins). Structural genomics initiatives may also benefit from this approach as a way to crystallize problematic proteins of significant interest.

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17428448 BIOSIS NO.: 200300385725
Cloning, expression and preliminary crystallographic studies of the potential drug target purine nucleoside phosphorylase from Schistosoma mansoni.
AUTHOR: Pereira Humberto M (Reprint); Cleasby Anne; Pena Sergio D J; Francoc Gloria R; Garratt Richard C
AUTHOR ADDRESS: Instituto de Fisica de Sao Carlos, Universidade de Sao Paulo, Sao Carlos, Brazil**Brazil
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JOURNAL: Acta Crystallographica Section D Biological Crystallography 59 (6): p1096-1099 June 2003 2003
MEDIUM: print
ISSN: 0907-4449
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The parasite Schistosoma mansoni, unlike its mammalian hosts, lacks the de novo pathway for purine biosynthesis and depends on salvage pathways for its purine requirements. The gene encoding one enzyme of this pathway, purine nucleoside phosphorylase from S. mansoni (SmPNP) was identified, fully sequenced and cloned into the bacterial expression vector pMAL c2G to produce a protein in %%%fusion%%% with maltose-%%%binding%%% %%%protein%%%. The recombinant %%%fusion%%% %%%protein%%% was expressed at high levels and was purified in a single step by amylose resin affinity chromatography. After factor Xa cleavage, SmPNP was purified using a cation-exchange column and crystallized by hanging-drop vapour diffusion using polyethylene glycol 1500 as precipitant in the presence of 20% glycerol in acetate buffer. The use of the non-detergent sulfobetaine 195 (NDSB 195) as an additive had a marked effect on the size of the resulting crystals. Two data sets were obtained, one from a crystal grown in the absence of NDSB 195 and one from a crystal grown in its presence. The crystals are isomorphous and belong to the space group P212121. It is intended to use the structures in the discovery and development of specific inhibitors of SmPNP.

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17366329 BIOSIS NO.: 200300324625
FUNCTIONS OF THE TIGHT JUNCTION PROTEIN OCCLUDIN: PHOSPHORYLATION AND

PROTEIN - PROTEIN INTERACTION WITH THE BLOOD - BRAIN BARRIER PROTEIN ZO - 1.
AUTHOR: Blasig I E (Reprint); Schmidt A (Reprint); Mller S L (Reprint); Andreeva A Y (Reprint); Utepbergenov D I (Reprint); Krause E (Reprint); Krause G (Reprint)
AUTHOR ADDRESS: Molecular Cell Physiology, Inst Molec Pharmacol, Berlin, Germany**Germany
JOURNAL: Society for Neuroscience Abstract Viewer and Itinerary Planner 2002 pAbstract No. 580.13 2002 2002
MEDIUM: cd-rom
CONFERENCE/MEETING: 32nd Annual Meeting of the Society for Neuroscience Orlando, Florida, USA November 02-07, 2002; 20021102
SPONSOR: Society for Neuroscience
DOCUMENT TYPE: Meeting; Meeting Poster; Meeting Abstract
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: THE TIGHT JUNCTION (TJ) PROTEIN OCCLUDIN (O): PHOSPHORYLATION AND INTERACTION WITH THE BLOOD-BRAIN BARRIER PROTEIN ZO-1 I.E. Blasig,* A. Schmidt, S.L. Mueller, A.Y. Andreeva, D.I. Utepbergenov, E. Krause, G. Krause. FMP, Berlin, Germany O is specific for TJ tightening the endothelium which forms the blood-brain barrier and regulates the TJ via its cytosolic C-terminal tail. The exact structure, function and regulation is unknown. O phosphorylation and interac-tion with the TJ-recruiting protein ZO-1 were studied. At low extracellular Ca2+, phorbol 12-myristate 13-acetate, a protein kinase C (PKC) stimulator, caused O phosphoryla-tion and transloca-tion into TJ, both augmented by PKC inhibition. The %%%fusion%%% %%%protein%%% MBP(maltose %%%binding%%% %%%protein%%%)-O.264-521 (mouse, complete cytosolic C-terminal part) was phosphorylated by PKC (Ser338: peptide mass fingerprint analysis, electrospray ionization tandem mass-spectroscopy). This provides the first direct proof that TJ are regulated via PKC. Using surface plasmon %%%resonance%%%, the protein binding region GuK-SH3 of ZO-1 (mouse MBP-ZO-1.589-772) was identified to bind to C-terminal O (aa 417-468). Association/dissociation showed moderate affinity and saturation kinetics within min: ka=4.14+/-0.52x10e3 1/Ms, kd=3.04+/-0.38x10e3 1/s, KD=639+/-51 nM. It is hypothesized that the SH3-GuK area of ZO-1 is involved in the attraction of ZO-1 to TJ if cells become confluent and phosphorylated O is incorporated into the TJ. The incorporation of ZO-1 to the TJ proceeds via the cytosolic C-terminal part of O after its Ca2+-dependent phosphorylation.

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17335202 BIOSIS NO.: 200300293021
VISUALIZATION AND MONITORING OF REGULATION OF CREB SIGNAL TRANSDUCTION PATHWAY IN LIVING CELLS BY FRET.
AUTHOR: Iwamoto T (Reprint); Kato K; Masushige S (Reprint); Kida S (Reprint)
AUTHOR ADDRESS: Department of Agricultural Chemistry, Tokyo Univ of Agriculture, Tokyo, Japan**Japan
JOURNAL: Society for Neuroscience Abstract Viewer and Itinerary Planner 2002 pAbstract No. 143.2 2002 2002
MEDIUM: cd-rom

CONFERENCE/MEETING: 32nd Annual Meeting of the Society for Neuroscience
Orlando, Florida, USA November 02-07, 2002; 20021102
SPONSOR: Society for Neuroscience
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The synthesis of new proteins plays an essential role in cAMP and Ca2+ signal transduction pathways are thought to regulate these gene expressions. PKA and CaMKs are activated by cAMP and Ca2+/CaM, respectively. These activated kinases phosphorylate transcription factor CREB at Serine 133. Phospho-CREB can interact with transcription coactivator CBP and then activate cAMP responsive element (CRE)-mediated transcription. Several studies using genetics indicated that CREB-dependent transcription is required for memory consolidation. However, precise molecular mechanisms underlying regulation of CRE-mediated transcription during the formation of LTM remain unknown. Therefore, we are trying to visualize and monitor key molecular interactions in the CREB signal transduction pathway. To detect protein-protein interaction in vivo, we measured fluorescence %%%resonance%%% energy transfer (FRET) using two %%%fusion%%% %%%protein%%% transiently expressed in PC12 cells: ECFP fused with CBP or CaM (CFP-CBP or -CaM) and EYFP fused with CREB or alphaCaMKII (YFP-CREB or -CaMKII). We monitored CaM-CaMKII or CREB-CBP interaction in living cells after stimulation. Our results showed that Ca2+ influx by ionomycin immediately induces CaM-CaMKII, and that activation of PKA by forskolin induces CBP-CREB interaction. We will present the results of real-time monitoring of these molecular interactions.

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17251252 BIOSIS NO.: 200300209971
Combinatorial crystallization of an RNA-protein complex.
AUTHOR: Hoggan Danielle Bodrero; Chao Jeffrey A; Prasad G S; Stout C David; Williamson James R (Reprint)
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JOURNAL: Acta Crystallographica Section D Biological Crystallography 59 (3): p466-473 March 2003 2003
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RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: One of the most difficult steps in X-%%%ray%%% crystallography of a ribonucleoprotein (RNP) complex is obtaining crystals that diffract to high resolution. This paper describes a procedure for identifying the optimal lengths of the nucleic acid components that provide high-quality crystals of the RNP. Both strands of an RNA duplex were varied in a systematic manner to generate a large number of unique RNPs that were screened for crystallization behavior. As observed in the crystallization of other nucleic acids and their complexes, the exact length of the RNA

chains was found to be critical in obtaining diffraction-quality crystals, even though the relative molecular weights of the protein and RNA components were apprx50 and apprx10 kDa, respectively. In particular, the helix-loop-helix structure in the mRNA for the Saccharomyces cerevisiae ribosomal protein L30, which functions as an autoregulatory element for L30 expression, was synthesized as two separate RNA chains of variable length (12-14 and 15-17 nucletides). Duplex formation of these RNAs formed the asymmetric, internal loop-binding site for L30. 16 such RNA duplexes, varying by +-1 residue at the 5' or 3' end of either chain, were used to prepare 16 unique complexes with a maltose-%%%binding%%% %%%protein%%%-L30 %%%fusion%%% %%%protein%%%. The complexes were screened against 48 standard crystallization conditions in 2304 experiments, yielding 30 conditions with single crystals in the initial screen. The most promising of these is being used for structure determination.

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17231976 BIOSIS NO.: 200300190695
Inherent protein structural flexibility at the RNA-binding interface of L30e.
AUTHOR: Chao Jeffrey A; Prasad G S; White Susan A; Stout C David; Williamson James R (Reprint)
AUTHOR ADDRESS: Department of Molecular Biology, Skaggs Institute for Chemical Biology, Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA, 92037, USA**USA
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JOURNAL: Journal of Molecular Biology 326 (4): p999-1004 28 February, 2003 2003
MEDIUM: print
ISSN: 0022-2836 _(ISSN print)
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The Saccharomyces cerevisiae ribosomal protein L30 autoregulates its own expression by binding to a purine-rich internal loop in its pre-mRNA and mRNA. NMR studies of L30 and its RNA complex showed that both the internal loop of the RNA as well as a region of the protein become substantially more ordered upon binding. A crystal structure of a maltose %%%binding%%% %%%protein%%% (MBP)-L30 %%%fusion%%% %%%protein%%% with two copies in the asymmetric unit has been determined. The flexible RNA-binding region in the L30 copies has two distinct conformations, one resembles the RNA bound form solved by NMR and the other is unique. Structure prediction algorithms also had difficulty accurately predicting this region, which is consistent with conformational flexibility seen in the NMR and X-%%%ray%%% crystallography studies. Inherent conformational flexibility may be a hallmark of regions involved in intermolecular interactions.

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17093884 BIOSIS NO.: 200300052603
Viroporin-mediated membrane permeabilization. Pore formation by nonstructural poliovirus 2B protein.
AUTHOR: Agirre Aitziber; Barco Angel; Carrasco Luis; Nieva Jose L (Reprint)
AUTHOR ADDRESS: Departamento de Bioquimica, Universidad del Pais Vasco, Aptdo. 644, Bilbao, 48080, Spain**Spain
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JOURNAL: Journal of Biological Chemistry 277 (43): p40434-40441 October 25, 2002 2002
MEDIUM: print
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Enterovirus nonstructural 2B protein is involved in cell membrane permeabilization during late viral infection. Here we analyze the pore forming activity of poliovirus 2B and several of its variants. Solubilization of 2B protein was achieved by generating a %%%fusion%%% %%%protein%%% comprised of poliovirus 2B attached to a maltose-%%%binding%%% %%%protein%%% (MBP) as an N-terminal solubilization partner. MBP-2B was assayed using large unilamellar vesicles as target membranes. This %%%fusion%%% %%%protein%%% was able to assemble into discrete structures that disrupted the permeability barrier of vesicles composed of anionic phospholipids. The transbilayer aqueous connections generated by MBP-2B were stable over time, allowing the passage of solutes of molecular mass under 1,000 Da. Oligomerization was investigated using fluorescence %%%resonance%%% energy transfer. Our data indicate that MBP-2B aggregation occurs at the membrane surface. Moreover, MBP-2B binding to membranes promoted the formation of SDS-resistant tetramers. We conclude that MBP-2B forms oligomers capable of generating a tetrameric aqueous pore in lipid bilayers. These findings are the first evidence of viroporin activity shown by a protein from a naked animal virus.

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16904847 BIOSIS NO.: 200200498358
Construction of gold-%%%binding%%% %%%protein%%%-vitamin D receptor %%%fusion%%% %%%protein%%% for surface plasmon %%%resonance%%% studies
AUTHOR: Lam CamAnh (Reprint); Collins Elaine D (Reprint)
AUTHOR ADDRESS: Department of Chemistry, San Jose State University, 129 So. Tenth St., San Jose, CA, 95192, USA**USA
JOURNAL: Abstracts of Papers American Chemical Society 223 (1-2): pCHED 297 2002 2002
MEDIUM: print
CONFERENCE/MEETING: 223rd National Meeting of the American Chemical Society Orlando, FL, USA April 07-11, 2002; 20020407
ISSN: 0065-7727
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Citation
LANGUAGE: English

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16867924 BIOSIS NO.: 200200461435
Imaging of metabolites by using a %%%fusion%%% %%%protein%%% between a periplasmic %%%binding%%% %%%protein%%% and GFP derivatives: From a chimera to a view of reality
AUTHOR: Stitt Mark (Reprint)
AUTHOR ADDRESS: Max Planck Institute of Molecular Plant Physiology, Am Muehlenberg 1, 14476, Golm, Germany**Germany
JOURNAL: Proceedings of the National Academy of Sciences of the United States of America 99 (15): p9614-9616 July 23, 2002 2002
MEDIUM: print
ISSN: 0027-8424
DOCUMENT TYPE: Article; Editorial
RECORD TYPE: Citation
LANGUAGE: English

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16652382 BIOSIS NO.: 200200245893
ALG-2 interacts with the amino-terminal domain of annexin XI in a Ca2+-dependent manner
AUTHOR: Satoh Hirokazu; Shibata Hideki; Nakano Yoshimi; Kitaura Yasuyuki; Maki Masatoshi (Reprint)
AUTHOR ADDRESS: Laboratory of Molecular and Cellular Regulation, Department of Applied Molecular Biosciences, Graduate School of Bioagricultural Sciences, Nagoya University, Furo-cho, Chikusa-ku, Nagoya, 464-8601, Japan**Japan
JOURNAL: Biochemical and Biophysical Research Communications 291 (5): p 1166-1172 March 15, 2002 2002
MEDIUM: print
ISSN: 0006-291X
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The apoptosis-linked protein ALG-2 is a Ca2+-%%%binding%%% %%%protein%%% that belongs to the penta-EF-hand protein family. ALG-2 forms a homodimer, a heterodimer with another penta-EF-hand protein, peflin, and a complex with its interacting protein, named AIP1 or Alix. By yeast two-hybrid screening using human ALG-2 as bait, we isolated a cDNA of a novel ALG-2-interacting protein, which turned out to be annexin XI. Deletion analysis revealed that ALG-2 interacted with the N-terminal domain of annexin XI (AnxN), which has an amino acid sequence similar to that of the C-terminal region of AIP1/Alix. Using recombinant biotin-tagged ALG-2 and the glutathione S-transferase (GST) %%%fusion%%% %%%protein%%% of AnxN, the direct interaction was analyzed by an ALG-2 overlay assay and by real-time interaction analysis with a surface plasmon %%%resonance%%% (SPR) biosensor. The dissociation constant (Kd) was estimated to be approximately 70 nM. The Ca2+-dependent fluorescence change of ALG-2 in the presence of the hydrophobicity fluorescent probe 2-p-toluidinylnaphthalene-6-sulfonate (TNS) was inhibited by mixing with

GST-AnxN, suggesting that the Pro/Gly/Tyr/Ala-rich hydrophobic region in AnxN masked the Ca2+-dependently exposed hydrophobic surface of ALG-2.

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16545426 BIOSIS NO.: 200200138937
Expression and purification of the extracellular domain of human myelin protein zero
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JOURNAL: Protein Expression and Purification 23 (3): p398-410 December, 2001 2001
MEDIUM: print
ISSN: 1046-5928
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Myelin protein zero (P0), an adhesion protein of the immunoglobulin superfamily, is the major protein of peripheral nervous system myelin in higher vertebrates. Protein zero is required for the formation and maintenance of myelin structure in the internode, likely through homophilic interactions at both the extracellular and the intracellular domains. Mutations and deletions in the P0 gene correlate with hereditary peripheral neuropathies of varying severity. Comparisons between the human and rat isoforms, whose three-dimensional structure has been determined by X-%%%ray%%% crystallography, suggest that these disease-associated genetic alterations lead to structural changes in the protein that alter P0-P0 interactions and hence affect myelin functionality. Knowing the crystal structures of native and altered human P0 isoforms could help to elucidate the structural changes in myelin membrane packing that underlie the altered functionality. Alterations of P0 extracellular domain (P0-ED) are of additional interest as previous X-%%%ray%%% diffraction studies on myelin membrane packing suggest that P0-ED molecules can assume distinct adhesive arrangements. Here, we describe an improved method to express and purify human P0-ED (hP0-ED) suitable for crystallographic analysis. A %%%fusion%%% %%%protein%%% consisting of maltose %%%binding%%% %%%protein%%% fused to hP0-ED was secreted to the periplasm of Escherichia coli to allow an appropriate folding pathway. The %%%fusion%%% %%%protein%%% was extracted via osmotic shock and purified by affinity chromatography. Factor Xa was used to cleave the %%%fusion%%% %%%protein%%%, and a combination of affinity and ion-exchange chromatography was used to further purify hP0-ED. We document several significant improvements to previous protocols, including bacterial growth to apprx15 OD using orbital shakers and the use of diafiltration, which result in yields of apprx150 mg highly pure protein per liter of medium.

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16248132 BIOSIS NO.: 200100419971
The functional unit of interphotoreceptor retinoid-%%%binding%%% %%%protein%%% (IRBP): Purification, characterization and preliminary crystallographic analysis
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JOURNAL: Experimental Eye Research 73 (2): p257-264 August, 2001 2001
MEDIUM: print
ISSN: 0014-4835
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: To define the relationship between the structure and function of interphotoreceptor retinoid-%%%binding%%% %%%protein%%% (IRBP) we seek to prepare crystals of IRBP suitable for X-%%%ray%%% crystallographic analysis. As recent studies suggest that each of IRBPs four homologous regions or modules possess ligand-binding activity, we here explore the feasibility of preparing crystals from an individual module. Xenopus laevis IRBP, which has a similar four-module structure as that of mammalian and avian IRBPs, was selected for these studies in view of the advantage of the Xenopus retina for cellular and transgenic approaches. In the present study we focused on the second module of Xenopus IRBP. This module was expressed as a thioredoxin/histidine-patch %%%fusion%%% %%%protein%%% to promote its soluble expression in Escherichia coli and subsequent purification. The ligand-binding properties of the %%%fusion%%% %%%protein%%% were determined by fluorescence spectroscopy. For the preparation of crystals, the module was enzymatically separated from the fusion tag. Crystals of the native and selenomethionine derivatized module were prepared by vapor diffusion in hanging drops. Module II of IRBP binds 1.57 +- 0.041 and 1.49 +- 0.15 equivalents of at all-trans retinol and 9-(9-anthroyloxy) stearic acid, respectively, with KDs in the 0.1 muM range. Crystals of this module had an elongated rectangular beam-like morphology. A complete dataset of a frozen selenomethionine crystal extending to 1.85 ANG resolution was collected. Focusing on the individual modules will likely provide an effective strategy to correlate biochemical and structural data to define the functional domains of IRBP. The quality and resolution of the data obtained suggests that it will be possible in the near future to solve the X-%%%ray%%% crystal structure of the IRBP modules.

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16182619 BIOSIS NO.: 200100354458
Crystal structure of the SarR protein from Staphylococcus aureus
AUTHOR: Liu Yingfang; Manna Adhar; Li Ronggui; Martin Wesley E; Murphy Robert C; Cheung Ambrose L; Zhang Gongyi (Reprint)
AUTHOR ADDRESS: 1400 Jackson Street, 501b, Denver, CO, 80206, USA**USA
JOURNAL: Proceedings of the National Academy of Sciences of the United States of America 98 (12): p6877-6882 June 5, 2001 2001
MEDIUM: print

ISSN: 0027-8424
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The expression of virulence determinants in Staphylococcus aureus is controlled by global regulatory loci (e.g., sarA and agr). The sar (Staphylococcus accessory regulator) locus is composed of three overlapping transcripts (sarA P1, P3, and P2, transcripts initiated from the P1, P3, and P2 promoters, respectively), all encoding the 124-aa SarA protein. The level of SarA, the major regulatory protein, is partially controlled by the differential activation of the sarA promoters. We previously partially purified a 13.6-kDa protein, designated SarR, that binds to the sarA promoter region to down-modulate sarA transcription from the P1 promoter and subsequently SarA expression. SarR shares sequence similarity to SarA, and another SarA homolog, SarS. Here we report the 2.3 ANG-resolution x-%%%ray%%% crystal structure of the dimeric SarR-MBP (maltose %%%binding%%% %%%protein%%%) %%%fusion%%% %%%protein%%%. The structure reveals that the SarR protein not only has a classic helix-turn-helix module for DNA binding at the major grooves, but also has an additional loop region involved in DNA recognition at the minor grooves. This interaction mode could represent a new functional class of the "winged helix" family. The dimeric SarR structure could accommodate an unusually long stretch of apprxeq27 nucleotides with two or four bending points along the course, which could lead to the bending of DNA by 90degree or more, similar to that seen in the catabolite activator protein (CAP)-DNA complex. The structure also demonstrates the molecular basis for the stable dimerization of the SarR monomers and possible motifs for interaction with other proteins.

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16118815 BIOSIS NO.: 200100290654
Analysis of opioid binding to UDP-glucuronosyltransferase 2B7 fusion proteins using nuclear magnetic %%%resonance%%% spectroscopy
AUTHOR: Coffman Birgit L; Kearney William R; Green Mitchell D; Lowery Robert G; Tephly Thomas R (Reprint)
AUTHOR ADDRESS: Department of Pharmacology, University of Iowa, BSB 2-452, Iowa City, IA, 52242, USA**USA
JOURNAL: Molecular Pharmacology 59 (6): p1464-1469 June, 2001 2001
MEDIUM: print
ISSN: 0026-895X
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The UDP-glucuronosyltransferase UGT2B7 is an important human UGT isoform that catalyzes the conjugation of many endogenous and exogenous compounds, among them opioids, resulting in the formation of D-glucuronides. The binding site of the aglycone is located in the N-terminal half of the protein. In this study, we demonstrate that the opioid binding site in UGT2B7 is within the first 119 amino-terminal amino acids. Two maltose %%%binding%%% %%%protein%%% fusion proteins, 2B7F1 and 2B7F2, incorporating the first 157 or 119 amino acids,

respectively, of UGT2B7 were expressed in Escherichia coli and purified by affinity chromatography. NMR spectroscopy using one-dimensional spectra, the inversion recovery method, and the transferred nuclear Overhauser effect spectroscopy was used to study the binding properties of opioids to the fusion proteins. Morphine was found to bind at a single site within the first 119 amino acids and to undergo a conformational change upon binding, as demonstrated by transferred nuclear Overhauser effect spectroscopy. Dissociation constants were obtained for morphine, naloxone, buprenorphine, and zidovudine, and the results were confirmed by equilibrium dialysis determinations. Two possible opioid binding sites, based on the nearest neighbors from opioid binding to the mu-receptor and to cytochrome 2D6, are proposed.

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16072957 BIOSIS NO.: 200100244796
Visualization and monitoring of CREB-CBP interaction in living cells by FRET
AUTHOR: Iwamoto Taku (Reprint); Asano Hidenori (Reprint); Aiba Yasuyuki (Reprint); Nakamura Takashi (Reprint); Masushige Shoichi (Reprint); Kida Satoshi (Reprint)
AUTHOR ADDRESS: Dep. of Agricultural Chemistry, Tokyo Univ. of Agriculture, Fuchu, Japan**Japan
JOURNAL: Neuroscience Research Supplement (24): pS31 2000 2000
MEDIUM: print
CONFERENCE/MEETING: 23rd Annual Meeting of the Japan Neuroscience Society and the 10th Annual Meeting of the Japanese Neural Network Society Yokohama, Japan September 04-06, 2000; 20000904
SPONSOR: Japan Neuroscience Society
Japanese Neural Network Society
ISSN: 0921-8696
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RECORD TYPE: Citation
LANGUAGE: English

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15991112 BIOSIS NO.: 200100162951
Identification and kinetic analysis of the interaction between Nck-2 and DOCK180
AUTHOR: Tu Yizeng; Kucik Dennis F; Wu Chuanyue (Reprint)
AUTHOR ADDRESS: Department of Pathology, University of Pittsburgh, 3550 Terrace Street, 707B Scaife Hall, Pittsburgh, PA, 15261, USA**USA
JOURNAL: FEBS Letters 491 (3): p193-199 2 March, 2001 2001
MEDIUM: print
ISSN: 0014-5793
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Nck-2 is a newly identified adapter protein comprising three

N-terminal SH3 domains and one C-terminal SH2 domain. We have identified in a yeast two-hybrid screen DOCK180, a signaling protein implicated in the regulation of membrane ruffling and migration, as a %%%binding%%% %%%protein%%% for Nck-2. Surface plasmon %%%resonance%%% analyses reveal that the second and the third SH3 domains interact with the C-terminal region of DOCK180. The interactions mediated by the individual SH3 domains, however, are much weaker than that of the full length Nck-2. Furthermore, a point mutation that inactivates the second or the third SH3 domain dramatically reduced the interaction of Nck-2 with DOCK180, suggesting that both SH3 domains contribute to the DOCK180 binding. A major Nck-2 binding site, which is recognized primarily by the third SH3 domain, has been mapped to residues 1819-1836 of DOCK180. Two additional, albeit much weaker, Nck-2 SH3 binding sites are located to DOCK180 residues 1793-1810 and 1835-1852 respectively. Consistent with the mutational studies, kinetic analyses by surface plasmon %%%resonance%%% suggest that two binding events with equilibrium dissociation constants of 4.15 +- 1.9 X 10-7 M and 3.24 +- 1.9 X 10-9 M mediate the binding of GST-Nck-2 to GST %%%fusion%%% %%%protein%%% containing the C-terminal region of DOCK180. These studies identify a novel interaction between Nck-2 and DOCK180. Furthermore, they provide a detailed analysis of a protein complex formation mediated by multiple SH3 domains revealing that tandem SH3 domains significantly enhance the weak interactions mediated by each individual SH3 domain.

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15979795 BIOSIS NO.: 200100151634
A study of protein-protein interactions in living cells using luminescence %%%resonance%%% energy transfer (LRET) from Renilla luciferase to Aequorea GFP
AUTHOR: Wang Y; Wang G; O'Kane D J; Szalay A A (Reprint)
AUTHOR ADDRESS: Department of Biochemistry, Loma Linda University, Loma Linda, CA, 92350, USA**USA
JOURNAL: Molecular and General Genetics 264 (5): p578-587 January, 2001 2001
MEDIUM: print
ISSN: 0026-8925
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: We have previously reported that Escherichia coli and mammalian cells containing a %%%fusion%%% %%%protein%%% consisting of the Renilla luciferase linked to Aequorea GFP exhibited luminescence %%%resonance%%% energy transfer (LRET) from luciferase to GFP in the presence of coelenterazine. In this paper, we describe the construction of two gene fusions in which the cDNA for insulin-like growth factor II (IGF-II) is connected to the cDNA for a "humanized" GFP, and the cDNA for insulin-like growth factor %%%binding%%% %%%protein%%% 6 (IGFBP-6) is linked to a cDNA encoding the Renilla luciferase (RUC). The expression of the fusion gene constructs in CHO cells resulted in single polypeptides with the molecular weights expected for IGF-II-GFP and IGFBP-6-RUC, respectively, based on the use of antibodies against GFP and Renilla luciferase. The secretion of IGF-II-GFP from CHO cells was verified by

fluorescence microscopy and the presence of IGFBP-6-RUC in the culture medium was confirmed by luminometry. The interaction between the two known binding partners, IGF-II and IGFBP-6, was monitored by measuring LRET from the IGFBP-6-RUC protein to IGF-II-GFP in the presence of coelenterazine, using a low-light imaging system and spectrofluorometry. Based on these data, luciferase-to-GFP LRET holds great promise for the study of protein-protein interactions in eukaryotic cells in real time.

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15943014 BIOSIS NO.: 200100114853
In vivo visualization of regulation and expression of CRE-mediated transcription during the formation of long-term memory
AUTHOR: Kida S (Reprint); Iwamoto T; Masushige S; Silva A J
AUTHOR ADDRESS: Tokyo University of Agriculture, Setagaya-ku, Tokyo, Japan **Japan
JOURNAL: Society for Neuroscience Abstracts 26 (1-2): pAbstract No.-652.4 2000 2000
MEDIUM: print
CONFERENCE/MEETING: 30th Annual Meeting of the Society of Neuroscience New Orleans, LA, USA November 04-09, 2000; 20001104
SPONSOR: Society for Neuroscience
ISSN: 0190-5295
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LANGUAGE: English

ABSTRACT: The synthesis of new proteins plays an essential role in LTM as well as long-term potentiation (LTP). Both cAMP and Ca2+ signal transduction pathways are thought to regulate these gene expressions. Members of the CREB/ATF families activate cAMP responsive element (CRE)-mediated transcription in response to increases in the intracellular concentration of cAMP and Ca2+. CREB is phosphorylated at serine 133 (S133) by PKA and CaMK. Phospho-CREB can then interact with transcription coactivator CBP. This molecular interaction is thought to be a crucial step in CREB-dependent transcription initiation. Using a traditional KO strategy (CREB-alpha/-DELTA), as well as with a inducible system (LBDG521R-CREBS133A), we showed that CREB-dependent transcription is required for formation of LTM. However, molecular mechanisms underlying CRE-mediated transcription during the formation of LTM remain unknown. Therefore, we are trying to visualize and monitor CRE-mediated transcription in vivo by using derivatives of the green fluorescent protein (GFP). To visualize the regulation of CRE-mediated transcription, we measured fluorescence %%%resonance%%% energy transfer (FRET) using two %%%fusion%%% %%%protein%%% transiently expressed in Cos1 cells (with or without PKA): enhanced cyan fluorescent protein (ECFP) fused with CBP (CFP-CBP) and enhanced yellow fluorescent protein (EYFP) fused with CREB (YFP-CREB). We detected evidence for an interaction between CFP-CBP and YFP-CREB that is dependent both on PKA and on CREB phosphorylation at S133. To monitor the CRE-mediated transcription activity, we have also derived transgenic mice with CRE-reporter gene. Enhanced green fluorescent protein (EGFP) and beta-galactosidase were used as reporter proteins. We will present the results of these analyses.

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15627091 BIOSIS NO.: 200000345404
A ligand-reversible dimerization system for controlling protein-protein interactions
AUTHOR: Rollins Carl T; Rivera Victor M; Woolfson Derek N; Keenan Terence; Hatada Marcos; Adams Susan E; Andrade Lawrence J; Yaeger David; van Schravendijk Marie Rose; Holt Dennis A; Gilman Michael; Clackson Tim (Reprint)
AUTHOR ADDRESS: ARIAD Gene Therapeutics, Inc., 26 Landsdowne Street, Cambridge, MA, 02139, USA**USA
JOURNAL: Proceedings of the National Academy of Sciences of the United States of America 97 (13): p7096-7101 June 30, 2000 2000
MEDIUM: print
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LANGUAGE: English

ABSTRACT: Chemically induced dimerization provides a general way to gain control over intracellular processes. Typically, FK506-%%%binding%%% %%%protein%%% (FKBP) domains are fused to a signaling domain of interest, allowing crosslinking to be initiated by addition of a bivalent FKBP ligand. In the course of protein engineering studies on human FKBP, we discovered that a single point mutation in the ligand-binding site (Phe-36 fwdarw Met) converts the normally monomeric protein into a ligand-reversible dimer. Two-hybrid, gel filtration, analytical ultracentrifugation, and x-%%%ray%%% crystallographic studies show that the mutant (FM) forms discrete homodimers with micromolar affinity that can be completely dissociated within minutes by addition of monomeric synthetic ligands. These unexpected properties form the basis for a "reverse dimerization" regulatory system involving FM fusion proteins, in which association is the ground state and addition of ligand abolishes interactions. We have used this strategy to rapidly and reversibly aggregate fusion proteins in different cellular compartments, and to provide an off switch for transcription. Reiterated FM domains should be generally useful as conditional aggregation domains (CADs) to control intracellular events where rapid, reversible dissolution of interactions is required. Our results also suggest that dimerization is a latent property of the FKBP fold: the crystal structure reveals a remarkably complementary interaction between the monomer binding sites, with only subtle changes in side-chain disposition accounting for the dramatic change in quaternary structure.

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15497694 BIOSIS NO.: 200000216007
Crystallization and preliminary X-%%%ray%%% diffraction analysis of the extracellular domain of the cell surface antigen CD38 complexed with ganglioside
AUTHOR: Kukimoto Mutsuko; Nureki Osamu; Shirouzu Mikako; Katada Toshiaki;

Hirabayashi Yoshio; Sugiya Hiroshi; Furuyama Shunsuke; Yokoyama Shigeyuki ; Hara-Yokoyama Miki (Reprint)
AUTHOR ADDRESS: Department of Physiology, Nihon University School of Dentistry at Matsudo, 2-870-1 Sakae-cho Nishi, Matsudo, 271-8587, Japan** Japan
JOURNAL: Journal of Biochemistry (Tokyo) 127 (2): p181-184 Feb., 2000 2000
MEDIUM: print
ISSN: 0021-924X
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The cell surface antigen CD38 is a multifunctional ectoenzyme that acts as an NAD+ glycohydrolase, an ADP-ribosyl cyclase, and also a cyclic ADP-ribose hydrolase. The extracellular catalytic domain of CD38 was expressed as a %%%fusion%%% %%%protein%%% with maltose-%%%binding%%% %%%protein%%%, and was crystallized in the complex with a ganglioside, GT1b, one of the possible physiological inhibitors of this ectoenzyme. Two different crystal forms were obtained using the hanging-drop vapor diffusion method with PEG 10,000 as the precipitant. One form diffracted up to 2.4 ANG resolution with synchrotron radiation at 100 K, but suffered serious X-%%%ray%%% damage. It belongs to the space group P212121 with unit-cell parameters of a = 47.9, b = 94.9, c = 125.2 ANG. The other form is a thin plate, but the data sets were successfully collected up to 2.4 ANG resolution by use of synchrotron radiation at 100 K. The crystals belong to the space group P21 with unit-cell parameters of a = 57.4, b = 51.2, c = 101.1 ANG, and beta = 97.9degree, and contain one molecule per asymmetric unit with a VM value of 2.05 ANG3/Da.

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15384762 BIOSIS NO.: 200000103075
Role of metallothionein in nitric oxide signaling as revealed by a green fluorescent %%%fusion%%% %%%protein%%%
AUTHOR: Pearce Linda L; Gandley Robin E; Han Weiping; Wasserloos Karla; Stitt Molly; Kanai Anthony J; McLaughlin Margaret K; Pitt Bruce R; Levitan Edwin S (Reprint)
AUTHOR ADDRESS: Department of Pharmacology, University of Pittsburgh, E1351 Biomedical Science Tower, Pittsburgh, PA, 15261, USA**USA
JOURNAL: Proceedings of the National Academy of Sciences of the United States of America 97 (1): p477-482 Jan. 4, 2000 2000
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RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Although the function of metallothionein (MT), a 6- to 7-kDa cysteine-rich metal %%%binding%%% %%%protein%%%, remains unclear, it has been suggested from in vitro studies that MT is an important component of intracellular redox signaling, including being a target for nitric oxide (NO). To directly study the interaction between MT and NO in live cells, we generated a %%%fusion%%% %%%protein%%% consisting of MT sandwiched between two mutant green fluorescent proteins (GFPs). In vitro studies

with this chimera (FRET-MT) demonstrate that fluorescent %%%resonance%%% energy transfer (FRET) can be used to follow conformational changes indicative of metal release from MT. Imaging experiments with live endothelial cells show that agents that increase cytoplasmic Ca2+ act via endogenously generated NO to rapidly and persistently release metal from MT. A role for this interaction in intact tissue is supported by the finding that the myogenic reflex of mesenteric arteries is absent in MT knockout mice (MT-/-) unless endogenous NO synthesis is blocked. These results are the first application of intramolecular green fluorescent protein (GFP)-based FRET in a native protein and demonstrate the utility of FRET-MT as an intracellular surrogate indicator of NO production. In addition, an important role of metal thiolate clusters of MT in NO signaling in vascular tissue is revealed.

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15296232 BIOSIS NO.: 200000014545
Identification and characterization of a Streptococcus pyogenes ABC transporter with multiple specificity for metal cations
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JOURNAL: Molecular Microbiology 34 (3): p596-606 Nov., 1999 1999
MEDIUM: print
ISSN: 0950-382X
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Metal ions are crucial trace elements for bacteria infecting the human host. The LraI (lipoprotein receptor-associated antigen I) transporter in Streptococcus spp. belongs to the superfamily of ABC transporters. The transporter consists of a lipoprotein, an ATP-%%%binding%%% %%%protein%%% and a hydrophobic integral membrane protein. Here, we describe a new member of the LraI family in the important human pathogen Streptococcus pyogenes. The system was identified in silico by analysis of the S. pyogenes Genome Sequencing Project. The S. pyogenes operon exhibits an atypical organization compared with equivalents in other Streptococcus spp. The presence and atypical organization of the operon was verified in a number of S. pyogenes strains of different serotypes. Transcriptional analysis of the LraI operon demonstrates a polycistronic transcription attenuated by a stable stem-loop structure, which allows the lipoprotein to be expressed in larger quantities than the other two components. The localization of the native lipoprotein at the bacterial surface was shown by proteolytic digestion of S. pyogenes bacteria and NH2-terminal sequencing of a released lipoprotein fragment. Recombinant lipoprotein was expressed as a GST %%%fusion%%% %%%protein%%% , and studies of molecular interactions with metal radioisotopes demonstrated that the protein has affinity for Zn(II), Fe(III) and Cu(II). Zn(II) and Cu(II) were found to compete for the same binding site, whereas Fe(III) uses a second site. Also, proton-induced X-%%%ray%%% analysis of lipoprotein samples identified iron, copper and zinc. Finally, a mutant strain lacking a functional mtsABC operon was generated and showed reduced uptake of 55Fe and 65Zn compared with the

wild-type strain. The operon encoding this novel ABC transporter with multiple specificity for metal cations is designated mtsABC, for metal transporter of Streptococcus.

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15216641 BIOSIS NO.: 199900476301
NMR analysis of the binding of a rhodanese peptide to a minichaperone in solution
AUTHOR: Kobayashi Naohiro; Freund Stefan M V; Chatellier Jean; Zahn Ralph; Fersht Alan R (Reprint)
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JOURNAL: Journal of Molecular Biology 292 (1): p181-190 Sept. 10, 1999 1999
MEDIUM: print
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RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: A detailed structural analysis of interactions between denatured proteins and GroEL is essential for an understanding of its mechanism. Minichaperones constitute an excellent paradigm for obtaining high-resolution structural information about the binding site and conformation of substrates bound to GroEL, and are particularly suitable for NMR studies. Here, we used transferred nuclear Overhauser effects to study the interaction in solution between minichaperone GroEL(193-335) and a synthetic peptide (Rho), corresponding to the N-terminal alpha-helix (residues 11 to 23) of the mitochondrial rhodanese, a protein whose in vitro refolding is mediated by minichaperones. Using a 60 kDa maltose-%%%binding%%% %%%protein%%% (MBP)-GroEL(193-335) %%%fusion%%% %%%protein%%% to increase the sensitivity of the transferred NOEs, we observed characteristic sequential and mid-range transferred nuclear Overhauser effects. The peptide adopts an alpha-helical conformation upon binding to the minichaperone. Thus the binding site of GroEL is compatible with binding of alpha-helices as well as extended beta-strands. To locate the peptide-binding site on GroEL(193-335), we analysed changes in its chemical shifts on adding an excess of Rho peptide. All residues with significant chemical shift differences are localised in helices H8 and H9. Non-specific interactions were not observed. This indicates that the peptide Rho binds specifically to minichaperone GroEL(193-335). The binding region identified by NMR in solution agrees with crystallographic studies with small peptides and with fluorescence quenching studies with denatured proteins.

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15102883 BIOSIS NO.: 199900362543
A high throughput assay of the hepatitis C virus nonstructural protein 3 serine proteinase

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JOURNAL: Journal of Virological Methods 80 (1): p77-84 June, 1999 1999
MEDIUM: print
ISSN: 0166-0934
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: A simple assay was developed based on intramolecular fluorescence %%%resonance%%% energy transfer for detection of the activity of hepatitis C virus (HCV) serine proteinase. Two quenched-flourogenic substrates, (7-methoxycoumarin-4-yl)acetyl (Mca) Asp-Asp-Ile-Val-Pro-Cys-Ser-Met-Ser-(2,4-dinitrophenyl, Dnp) Lys (Mca-Asp-Asp-Ile-Val-Pro-Cys-Ser-Met-Ser-Lys(Dnp), QF-1) and Mca-Asp-Asp-Ile-Val-Pro-Cys-Ser-Met-Lys(Dnp)-Arg-Arg (QF-2), which derived from the NS5A/5B junction of the HCV polyprotein, were designed. Kinetic studies revealed that QF-1 and QF-2 had high affinity for a recombinant enzyme which is a %%%fusion%%% %%%protein%%% of maltose %%%binding%%% %%%protein%%% and almost entire nonstructural protein (MBP-NS3), with Km values comparable to that of longer substrate based on the same cleavage site. QF-1 and QF-2 were cleaved by MBP-NS3 efficiently with kcat values of 7.5 and 4.2 min-1, respectively. QF-2 was also found to be a good substrate of DELTANS3 which contained serine proteinase part of NS3 with kcat value of 4.3 min-1. The cleavage reaction is detected continuously by the elevation of the fluorescence due to release from quenching. The fluorescence of the substrates increases in proportion to progress of the cleavage reaction under the standard conditions. This method was applied for screening of HCV serine protease inhibitors using a fluorescence multiwell plate reader. A group of natural occurring products, flavonoids, was subjected to be screened. Two flavonoids out of 25 were found to inhibit the enzyme moderately at a concentration of 100 muM. The data agreed with those obtained by high-performance liquid chromatography (HPLC). This method is suited to sensitive quantitation of the enzyme reaction as well as the high throughput analysis of the inhibitors.

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14948672 BIOSIS NO.: 199900208332
Structure of the enzymatically synthesized fructan inulin
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JOURNAL: Carbohydrate Research 313 (3-4): p165-174 Dec. 15, 1998 1998
MEDIUM: print
ISSN: 0008-6215
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RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Construction, purification and characterization of a %%%fusion%%% %%%protein%%% of maltose-%%%binding%%% %%%protein%%% of Escherichia coli and the fructosyltransferase of Streptococcus mutans is described. With the purified protein, in vitro synthesis of inulin was performed. The obtained polysaccharide was characterized by high-performance size-exclusion chromatography (HPSEC) and static light scattering (SLS) in dilute aqueous and dimethyl sulfoxide solution. For all samples very high molecular weights between 60 X 106 and 90 X 106 g/mol and a remarkable small polydispersity index of 1.1 have been determined. Small root-mean-square radii of gyration point to a compact conformation in dilute solution. No difference between native and enzymatically synthesized inulin was observed by X-%%%ray%%% powder diffraction and thermoanalysis of solid samples.

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14710846 BIOSIS NO.: 199800505093
The maltose-%%%binding%%% %%%protein%%% as a scaffold for monovalent display of peptides derived from phage libraries
AUTHOR: Zwick Michael B; Bonnycastle Lori L C; Noren Karen A; Venturini Sara; Leong Edward; Barbas Carlos F III; Noren Christopher J; Scott Jamie K (Reprint)
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JOURNAL: Analytical Biochemistry 264 (1): p87-97 Nov. 1, 1998 1998
MEDIUM: print
ISSN: 0003-2697
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Random peptide libraries are displayed on filamentous bacteriophage as fusions to either the minor coat protein, pIII, or the major coat protein, pVIII. We have devised a means of isolating the peptide displayed on a phage clone by transferring it to the N-terminus of the maltose-%%%binding%%% %%%protein%%% (MBP) of Escherichia coli encoded by malE. Transfer of a peptide sequence to monomeric MBP eliminates phage-encoded amino acids downstream of the insert peptide as well as avidity effects caused by multivalent display on phage. Peptide:MBP fusions are also easily affinity purified on amylose columns. The pMal-p2 vector was engineered to accept phage DNA encoding pIII- and pVIII-displayed peptides fused to their respective leader sequences. Both types of leader sequence were shown to target the peptide:MBP fusions to the periplasm of E. coli. A streamlined procedure for transferring peptides to MBP was applied to clones that had been isolated from a panel of pVIII-displayed peptide libraries by screening with an HIV-1-specific monoclonal antibody (Ab). By enzyme-linked immunosorbent assay, the Ab bound each of the peptide:MBP fusions and required the presence of a disulfide bridge within each peptide. Some of the peptide:MBP fusions were also analyzed using surface plasmon %%%resonance%%%. Thus, our study shows the value of male fusion vectors in characterizing phage-displayed peptides.

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14678658 BIOSIS NO.: 199800472905
Crystallization of a trimeric human T cell leukemia virus type 1 gp21 ectodomain fragment as a %%%chimera%%% with maltose-%%%binding%%% %%%protein%%%
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JOURNAL: Protein Science 7 (7): p1612-1619 July, 1998 1998
MEDIUM: print
ISSN: 0961-8368
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: We present a novel protein crystallization strategy, applied to the crystallization of human T cell leukemia virus type 1 (HTLV-1) transmembrane protein gp21 lacking the fusion peptide and the transmembrane domain, as a chimera with the Escherichia coli maltose %%%binding%%% %%%protein%%% (MBP). Crystals could not be obtained with a MBP/gp21 %%%fusion%%% %%%protein%%% in which fusion partners were separated by a flexible linker, but were obtained after connecting the MBP C-terminal alpha-helix to the predicted N-terminal alpha-helical sequence of gp21 via three alanine residues. The gp21 sequences conferred a trimeric structure to the soluble fusion proteins as assessed by sedimentation equilibrium and X-%%%ray%%% diffraction, consistent with the trimeric structures of other retroviral transmembrane proteins. The envelope protein precursor, gp62, is likewise trimeric when expressed in mammalian cells. Our results suggest that MBP may have a general application for the crystallization of proteins containing N-terminal alpha-helical sequences.

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14507948 BIOSIS NO.: 199800302195
Isolation and characterization of pediocin AcH %%%chimeric%%% %%%protein%%% mutants with altered bactericidal activity
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JOURNAL: Applied and Environmental Microbiology 64 (6): p1997-2005 June, 1998 1998
MEDIUM: print
ISSN: 0099-2240
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RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: A collection of pediocin AcH amino acid substitution mutants was

generated by PCR random mutagenesis of DNA encoding the bacteriocin. Mutants were isolated by cloning mutagenized DNA into an Escherichia coli malE plasmid that directs the secretion of maltose %%%binding%%% %%%protein%%%-pediocin AcH chimeric proteins and by screening transformant colonies for bactericidal activity against Lactobacillus plantarum NCDO955 (K. W. Miller, R. Schamber, Y. Chen, and B. %%%Ray%%%, 1998. Appl. Environ. Microbiol. 64:14-20, 1998). In all, 17 substitution mutants were isolated at 14 of the 44 amino acids of pediocin AcH. Seven mutants (N5K, C9R, C14S, C14Y, G37E, G37R, and C44W) were completely inactive against the pediocin AcH-sensitive strains L. plantarum NCDO955, Listeria innocua Lin11, Enterococcus faecalis M1, Pediococcus acidilactici LB42, and Leuconostoc mesenteroides Ly. A C24S substitution mutant constructed by other means also was inactive against these bacteria. Nine other mutants (K1N, W18R, 126T, M31T, A34D, N41K, H42L, K43N, and K43E) retained from < 1% to apprx60% of wild-type activity when assayed against L. innocua Lin11. One mutant, K11E, displayed apprx 2.8-fold-higher activity against this indicator. About one half of the mutations mapped to amino acids that are conserved in the pediocin-like family of bacteriocins. All four cysteines were found to be required for activity, although only C9 and C14 are conserved among pediocin-like bacteriocins. Several basic amino acids as well as nonpolar amino acids located within the hydrophobic C-terminal region also were found to be important. The mutations are discussed in the context of structural models that have been proposed for the bacteriocin.

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13770558 BIOSIS NO.: 199799404618
Expression, purification and characterization of recombinant crambin
AUTHOR: Lobb Leslie; Stec Boguslaw; Kantrowitz Evan K; Yamano Akihito; Stojanoff Vivian; Markman Ofer; Teeter Martha M (Reprint)
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JOURNAL: Protein Engineering 9 (12): p1233-1239 1996 1996
ISSN: 0269-2139
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RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Crambin, a small hydrophobic protein (4.7 kDa and 46 residues), has been successfully expressed in Escherichia coli from an artificial, synthetic gene. Several expression systems were investigated. Ultimately, crambin was successfully expressed as a %%%fusion%%% %%%protein%%% with the maltose %%%binding%%% %%%protein%%%, which was purified by affinity chromatography. Crambin expressed as a C-terminal domain was then cleaved from the %%%fusion%%% %%%protein%%% with Factor Xa protease and purified. Circular dichroism spectroscopy and amino acid analysis suggested that the purified material was identical to crambin isolated from seed. For positive identification the protein was crystallized from an ethanol-water solution, by a novel method involving the inclusion of phospholipids in the crystallization buffer, and then subjected to crystallographic analysis. Diffraction data were collected at the Brookhaven synchrotron (beamline-X12C) to a resolution of 1.32 ANG at 150 K. The structure, refined to an R value of 9.6%, confirmed that the

cloned protein was crambin. The availability of cloned crambin will allow site-specific mutagenesis studies to be performed on the protein known to the highest resolution.

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13264985 BIOSIS NO.: 199698732818
Crystallization and preliminary X-%%%ray%%% analysis of twinned crystals of a %%%chimeric%%% FK506 %%%binding%%% %%%protein%%% 12 and 13 complexed with FK506
AUTHOR: Liang Jun; Ealick Steve; Nielsen Chris; Schreiber Stuart L; Clardy Jon (Reprint)
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JOURNAL: Acta Crystallographica Section D Biological Crystallography 52 (1): p207-210 1996 1996
ISSN: 0907-4449
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: An FKBP12/13 chimera with the 80s loop of FKBP13 replacing the corresponding loop in FKBP12 tightly binds the immunosuppressive agents FK506 and rapamycin and efficiently catalyzes peptidyl-prolyl cis-trans isomerization. However, the chimera's complex with FK506 does not inhibit calcineurin's phosphatase activity (Yang, Rosen and Schreiber (1993). J. Am. Chem. Soc. 115(2), 819-820). The %%%chimeric%%% %%%protein%%% crystallizes in space group PI and the crystals are always twinned. The twin composites are related by a twofold twinning axis parallel to the a axis. A resolution data set (1.5 ANG resolution) for a twinned crystal was collected at CHESS using 0.91 ANG X-rays and image plates. Preliminary molecular replacement using data between 15 and 3 ANG and the FKBP12-FK506 crystal structure as the search model led to a clear solution with a residual of 34.2%. This 3 ANG resolution structure provides insight into the structural basis of twinning.

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13175142 BIOSIS NO.: 199698642975
Cloning of cockroach allergen, Bla g 4, identifies ligand binding proteins (or calycins) as a cause of IgE antibody responses
AUTHOR: Arruda L Karla; Vailes Lisa D; Hayden Mary L; Benjamin David C; Chapman Martin D (Reprint)
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JOURNAL: Journal of Biological Chemistry 270 (52): p31196-31201 1995 1995
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: An allergen cloned from a Blattella germanica (German cockroach)

cDNA library, encoded a 182-amino acid protein of 20,904 Da. This protein, designated B. germanica allergen 4 (Bla g 4), was expressed as a glutathione S-transferase %%%fusion%%% %%%protein%%% in Escherichia coli and purified by affinity chromatography and high-performance liquid chromatography. The prevalence of serum IgE antibody to recombinant Bla g 4 in 73 cockroach allergic patients with asthma ranged from 40% (antigen binding radioimmunoassay) to 60% (plaque immunoassay). Cockroach allergic patients gave positive intradermal skin tests to recombinant Bla g 4 at concentrations of 10-3-10-5 mu-g/ml, whereas non-allergic controls, or cockroach allergic patients with no detectable serum IgE antibody to Bla g 4, gave negative skin tests to 1 mu-g/ml. Polymerase chain reaction and Southern analysis identified a 523-base pair DNA encoding Bla g 4 in both B. germanica and Periplaneta americana (American cockroach). However, Northern analysis showed that mRNA encoding Bla g 4 was transcribed in B. germanica but not in P. americana, suggesting that allergen expression was species specific. Sequence similarity searches showed that Bla g 4 was a ligand %%%binding%%% %%%protein%%% or calycin and unexpectedly revealed that this family contained several important allergens: beta-lactoglobulin, from cow milk, and rat and mouse urinary proteins. Although the overall sequence homology between these proteins was low (apprx 20%), macromolecular modeling techniques were used to generate two models of the tertiary structure of Bla g 4, based on comparisons with the x-%%%ray%%% crystal coordinates of bilin %%%binding%%% %%%protein%%% and rodent urinary proteins. The results show that members of the calycin protein family can cause IgE antibody responses by inhalation or ingestion and are associated with asthma and food hypersensitivity.

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12168983 BIOSIS NO.: 199497190268
Structural organization of the gene encoding the human lipocalin tear prealbumin and synthesis of the recombinant protein in Escherichia coli
AUTHOR: Holzfeind Paul; Redl Bernhard (Reprint)
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JOURNAL: Gene (Amsterdam) 139 (2): p177-183 1994 1994
ISSN: 0378-1119
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The genomic DNA fragment encoding the human lipocalin tear prealbumin (LCN1), a new member of the superfamily of hydrophobic molecule transporters, has been isolated and sequenced. The entire gene is approximately 6.2 kb in size and contains six protein-coding exons and a 3'-nontranslated exon. All exon/intron splice junctions exactly follow the GT/AG rule. The structure of the LCN1 gene is highly similar, in terms of numbers and sizes of exons and in intron phasing, to that of the genes encoding ovine beta-lactoglobulin, human placental protein P14, rat alpha-2-urinary globulin, rat prostaglandin D synthase and human alpha-1-microglobulin, thus supporting the close evolutionary relationship of these genes. The 5'-noncoding region of LCN1 contains, besides a TATA and CAAT box, several motifs that resemble regulatory elements of other eukaryotic genes, including potential metal-responsive

elements (MRE) and a cAMP-responsive element (CRE). As a basis for further investigations concerning the structure-function relationship and to generate a source of recombinant protein for X-%%%ray%%% crystallography studies, LCN1 was produced in Escherichia coli as a %%%fusion%%% with maltose-%%%binding%%% %%%protein%%%.

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18181732 BIOSIS NO.: 200500088797
P-selectin-targeting of the fibrin selective thrombolytic Desmodus rotundus salivary plasminogen activator at alpha1
AUTHOR: Dong Ningzheng (Reprint); Da Cunha Valcleci; Citkowicz Andrej; Wu Faye; Vincelette Jon; Larsen Brent; Wang Yi-Xin; Ruan Changgeng; Dole William P; Morser John; Wu Qingyu; Pan Junliang
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JOURNAL: Thrombosis and Haemostasis 92 (5): p956-965 November 2004 2004
MEDIUM: print
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LANGUAGE: English

ABSTRACT: During thrombosis, P-selectin is expressed on the surface of activated endothelial cells and platelets. We hypothesized that targeting a plasminogen activator (PA) to P-selectin would enhance local thrombolysis and reduce bleeding risk. Previously, a urokinase (uPA)/anti-P-selectin antibody (HuSZ51) fusion protein was shown to increase fibrinolysis in a hamster pulmonary embolism model. To explore the therapeutic potential of this targeting strategy, we fused the fibrin-selective Desmodus rotundus salivary PA alpha1 (dsPAalpha1) to HuSZ51 and compared the fibrinolytic activity of P-selectin-targeted dsPAalpha1 (HuSZ51-dsPAalpha1) to unmodified dsPAalpha1 in vitro and in vivo. HuSZ51-dsPAalpha1 and dsPAalpha1 were expressed in CHO cells and purified to homogeneity by affinity chromatography. HuSZ51-dsPAalpha1 bound to thrombin-activated human and dog platelets with comparable affinities to that of parental antibody SZ51. The %%%fusion%%% %%%protein%%% retained the catalytic activities of dsPAalpha1 in %%%chromogenic%%% and clot lysis assays, indicating that dsPAalpha1 is fully functional when fused to HuSZ51. Compared to dsPAalpha1, HuSZ51-dsPAalpha1 had similar thrombolytic efficacy in a rat pulmonary embolism model and anti-thrombotic potency in a dog model of femoral artery thrombosis. However, HuSZ51-dsPAalpha1 was less effective in lysis of preexisting arterial thrombi in the dog model. The reduced arterial thrombolysis was not due to the pharmacokinetic properties of HuSZ51-dsPAalpha1 because antigen level and amidolytic activity were higher in plasma from HuSZ51-dsPAalpha1-treated groups than corresponding dsPAalpha1-treated groups. These data indicate that the thrombolytic efficacy of HuSZ51-dsPAalpha1 varied dependent on the physical composition of thrombi. The lack of stimulation by fibrin in arterial thrombi may contribute to the attenuated thrombolytic efficacy of

HuSZ51-dsPAalpha1 in the dog model.

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19183756 BIOSIS NO.: 200600529151
Structure-activity profiles of ab-derived TNF fusion proteins
AUTHOR: Bauer Stefan (Reprint); Adrian Nicole; Fischer Eliane; Kleber Sascha; Stenner Frank; Wadle Andreas; Fadle Natalie; Zoellner Andy; Bernhardt Rita; Knuth Alexander; Old Lloyd J; Renner Christoph
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JOURNAL: Journal of Immunology 177 (4): p2423-2430 AUG 15 2006 2006
ISSN: 0022-1767
DOCUMENT TYPE: Article
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LANGUAGE: English

ABSTRACT: TNF application in humans is limited by severe side effects, including life-threatening symptoms of shock. Therefore, TNF can be successfully applied as a tumor therapeutic %%%reagent%%% only under conditions that prevent its systemic action. To overcome this limitation, genetic fusion of TNF to tumor-selective Abs is a favored strategy to increase site-specific cytokine targeting. Because wild-type TNF displays its bioactivity as noncovalently linked homotrimer, the challenge is to define structural requirements for a TNF-based immunokine format with optimized structure-activity profile. We compared toxicity and efficacy of a dimerized CH2/CH3 truncated IgG1-TNF fusion protein and a single-chain variable fragment-coupled. TNF monomer recognizing fibroblast-activating protein. The former construct preserves its dimeric structure stabilized by the natural disulfide bond IgG1 hinge region, while the latter trimerizes under native conditions. Analysis of complex formation of wild-type TNF and of both fusion proteins with TNFR type 1 (TNF-R1) using surface plasmon %%%resonance%%% correlated well with in vitro and in vivo toxicity data. There is strong evidence that TNF subunits in a trimeric state display similar toxicity profiles despite genetic fusion to single-chain variable fragment domains. However, LD50 of either immunodeficient BALB/c nu/nu or immunocompetent BALB/c mice was significantly decreased following %%%administration%%% of TNF in the formation of IgG1-derived dimeric fusion protein. Reduction of unspecific peripheral complexation of TNF-R1 resulted in higher anticancer potency by immunotargeting of fibroblast-activating protein-expressing xenografts. The broader therapeutic window of the IgG1-derived TNF fusion protein favors the dimeric TNF-immunokine format for systemic TNF-based tumor immunotherapy.

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18937979 BIOSIS NO.: 200600283374
An ESR contrast agent is transported to rat liver through organic anion transporter
AUTHOR: Ichikawa Kazuhiro; Sato Yasuko; Kondo Hiroshi; Utsumi Hideo (Reprint)
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JOURNAL: Free Radical Research 40 (4): p403-408 APR 2006 2006
ISSN: 1071-5762
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Carboxy PROXYL is a useful extracellular paramagnetic contrast %%%reagent%%% in electron spin %%%resonance%%% (ESR) and magnetic %%%resonance%%% imaging (MRI). Active transfer of the probe was investigated using an in situ liver model in rats. Carboxy PROXYL, a nitroxyl spin probe, was perfused into in situ liver perfusion system from Wistar rats. Concentration of nitroxyl form of the spin probe in effluent increased gradually after introducing perfusate with the spin probe and reached a plateau. The disappearance of Carboxy PROXYL from the perfusate was 40%, which could not be explained with its partition coefficient. %%%Administration%%% of non-selective inhibitors of organic anion transporters, p-aminohippuric acid and penicillin G, inhibited competitively and in a dose dependent manner the transfer of Carboxy PROXYL into rat liver in situ, resulting in increases of Carboxy PROXYL in the effluent. The results demonstrate that there is an active transfer system of an ESR contrast %%%reagent%%% into in situ rat liver through organic anion transporters.

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18748621 BIOSIS NO.: 200600094016
Free radical generation and lipid peroxidation induced by 2, 4-dichlorophenol in liver of Carassius auratus
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LANGUAGE: Chinese

ABSTRACT: Free radical generation in liver of larval fish (Carassius auratus) after 2, 4-dichlorophenol (2, 4-DCP) injected intraperitoneally24h was studied with electron paramagnetic %%%resonance%%%(EPR) spin trapping technique and alpha-phenyl-N-tert-butylnitrone(PBN) as spin trap %%%reagent%%%. The results show that there is a significant increase of free radical generation in the liver of the fish treated with 2,4-DCP(0.025, 0.05, 0.5, 5, 25 mg/kg) compared with that of the control($p<0.05$). The free

radical generation correlates well with the dose of 2,4-DCP %%%administered%%%. The regress equation for dose-effect relation is y=11891n(x) + 9220, r(2)=0.9856. The six-line composed of three groups with two hyperfine splitting peaks in each shows a typical PBN/OH EPR spectrum. The hyperfine splitting constants for the PBN-adducts is aN=13.7 Gauss, aH=1.8 Gauss, g factor is 2.0058, which is consistent with the characteristics of PBN-hydroxyl radical adduct reported from the literature. Results also show that there is a significant increase for malondialdehyde(MDA) content in the treated groups compared with that of the control($p<0.05$). The mechanisms of free radical generation and lipid peroxidation induced by 2,4-DCP are discussed in this study.

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18316435 BIOSIS NO.: 200510010935
Synthesis of well-defined amphiphilic block copolymers having phospholipid polymer sequences as a novel blocompatible polymer micelle %%%reagent%%%
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JOURNAL: Biomacromolecules 6 (2): p663-670 MAR-APR05 2005
ISSN: 1525-7797
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LANGUAGE: English

ABSTRACT: To realize safer and effective drug %%%administration%%%, novel well-defined and biocompatible amphiphilic block copolymers containing phospholipid polymer sequences were synthesized. At first, the homopolymer of 2-methacryloyloxyethylphosphorylcholine (MPC) was synthesized in water by reversible addition-fragmentation chain transfer (RAFT) controlled radical polymerization. The "living" polymerization was confirmed by the fact that the number-average molecular weight increased linearly with monomer conversion while the molecular weight distribution remained narrow independent of the conversion. The poly(MPC) thus prepared is end-capped with a dithioester moiety. Using the dithioester-capped poly(MPC) as a macro chain transfer agent, AB diblock copolymers of MPC and n-butyl methacrylate (BMA) were synthesized. Associative properties of the amphiphilic block copolymer (pMPC(m)-BMA(n)) with varying poly(BMA) block lengths were investigated using NMR, fluorescence probe, static light scattering (SLS), and quasi-elastic light scattering (QELS) techniques. Proton NMR data in D2O indicated highly restricted motions of the n-butyl moieties, arising from hydrophobic associations of poly(BMA) blocks. Fluorescence spectra of N-phenyl-1-naphthylamine indicated that the probes were solubilized in the polymer micelles in water. The formation of polymer micelles comprising a core with poly(BMA) blocks and shell with hydrophilic poly(MPC) blocks was suggested by SLS and QELS data. The size and mass of the micelle increased with increasing poly(BMA) block length. With an expectation of a pharmaceutical application of pMPC(m)-BMA(n), solubilization of a poorly water-soluble anticancer agent, paclitaxel (PTX), was investigated. PTX dissolved well in aqueous solutions of

pMPC(m)-BMA(n) as compared with pure water, implying that PTX is incorporated into the hydrophobic core of the polymer micelle. Since excellent biocompatible poly(MPC) sequences form an outer shell of the micelle, pMPC(m)-BMA(n) may find application as a promising %%%reagent%%% to make a good formulation with a hydrophobic drug.

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17901895 BIOSIS NO.: 200400272652
Model for predicting the of gadolinium on plasma calcium measured by the o-cresolphthalein method
AUTHOR: Kang Hyunseok P; Scott Mitchell G; Joe Bonnien N; Narra Vamsi; Heiken Jay; Parvin Curtis A (Reprint)
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JOURNAL: Clinical Chemistry 50 (4): p741-746 April 2004 2004
MEDIUM: print
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RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Background: Gadolinium formulations, which are %%%administered%%% as contrast agents in magnetic %%%resonance%%% imaging examinations, interfere with colorimetric serum calcium determinations. Methods: We performed-an in vitro study to determine the extent to which three, gadolinium formulations-gadodiamide (Omniscan), gadopentetate dimeglumine (Magnevist), and gadoversetamide (OptiMARK)-affect. measurements by two methods that use o-cresolphthalein (Dade Behring, Inc. and Roche Diagnostics) and one that uses arsenazo dye (Equal Diagnostics). We also compared values from the, o-cresolphthalein methods for 116 samples from patients %%%administered%%% gadodiamide. Results: Magnevist did not affect any of the methods evaluated, whereas Omniscan and OptiMARK were identical in their effects. For the Dade method, the differences from the control sample were ltoreq4.0 and 7.0 mg/L at 0.25 and 0.5 mmol/L gadolinium, respectively. For the Roche method, the differences were 19, 9.0, and 5.0 mg/L at 0.5, 0.25, and 0.125 mmol/L gadolinium,. respectively. Falsely increased calcium values were seen, when samples were measured by the arsenazo-based method: differences were 6.0 and 3.0 mg/L at 1.0 and 0.5 mmol/L gadolinium. Using patient data collected at our institution, we were able to generate a model for predicting, from a patient's glomerular filtration rate and the time elapsed since %%%administration%%%, the impact of Omniscan on calcium measurements by the o-cresolphthalein method from Roche Diagnostics. Conclusions: The predictive model can be used to calculate, in patients who have received gadodiamide, the minimum length of time to wait before blood collection to avoid pseudohypocalcemia when the, Roche o-cresolphthalein method is used. Copyright 2004 American Association for Clinical Chemistry.

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16340904 BIOSIS NO.: 200100512743
Neuropeptide Y and its receptor analogs modulate ischemia-induced nitric oxide production
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JOURNAL: Society for Neuroscience Abstracts 27 (1): p875 2001 2001
MEDIUM: print
CONFERENCE/MEETING: 31st Annual Meeting of the Society for Neuroscience San Diego, California, USA November 10-15, 2001; 20011110
ISSN: 0190-5295
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LANGUAGE: English

ABSTRACT: We previously showed that intracerebroventricular (ICV) injection of neuropeptide Y (NPY) or Y1 agonist increased the infarct volume whereas %%%administration%%% of BIBP3226, a NPY Y1 antagonist, reduced the infarct volume. Nitric oxide (NO) is a mediator of ischemic damage. In this study, we examined the effects of NPY and its receptor analogs on NO production during middle cerebral artery occlusion. Male Sprague-Dawley rats were anesthetized with sodium pentobarbital. NPY (10 mcg/kg), (Leu31, Pro34)-NPY (30 mcg/kg), BIBP3226 (15 mcg/kg), NPY3-36 (15 mcg/kg) or vehicle was %%%administered%%% via a slow ICV injection at 2 minutes after onset of ischemia. NO measurement was made in the brain slices between Bregma levels +2 and -4 mm. NO trapping reagents, diethyldithiocarbamate and iron citrate, were %%%administered%%% via intraperitoneal and subcutaneous injection, respectively, at 15 minutes prior to ischemia. Tissue NO concentration was measured using electron paramagnetic %%%resonance%%% spectroscopy. Results from the ischemic side were expressed as a percentage of the non-ischemic side and compared among groups using two-tailed Student's test. After 15 minutes of focal cerebral ischemia, the relative NO concentration increased to 131.9+-8.0% (mean+-SEM; n=8). NPY treatment further increased the NO signal (250.9+-50.5%; n=8, P<0.05), whereas BIBP3226 reduced the NO signal (69.6+-8.8%; n=8, P<0.05). Treatment with (Leu31, Pro34)-NPY and NPY3-36 did not affect the ischemia-induced NO signal (133.4+-13.3%, n=8; 129.2+-21.8%, n=8). Thus, exogenous NPY and its receptor analogs may affect the infarct volume via their effects on ischemic-induced NO generation.

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16226040 BIOSIS NO.: 200100397879
Demonstration of the decreased mitochondrial reducing activity in puromycin aminonucleoside nephrosis using an electron spin %%%resonance%%% technique
AUTHOR: Ueda A (Reprint); Nagase S (Reprint); Hirayama A (Reprint); Aoyagi K (Reprint); Koyama A (Reprint); Yokoyama H; Ohya H; Kamada H
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JOURNAL: Nephrology Dialysis Transplantation 16 (6): pA39 June, 2001 2001
MEDIUM: print

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SPONSOR: European Renal Association
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16158938 BIOSIS NO.: 200100330777
The monitoring of heparin %%%administration%%% by screening tests in experimental dogs
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JOURNAL: Research in Veterinary Science 70 (2): p101-108 April, 2001 2001
MEDIUM: print
ISSN: 0034-5288
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RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The objective of this study was to investigate the relationship between different screening tests of haemostasis and amidolytic plasma activities of unfractionated (standard) heparin in dogs. Different doses of intravenous (i.v.) (25, 50 or 100 IU Kg-1 body-weight (BW)) and subcutaneous (s.c.) heparin (250, 500 and 750 IU kg-1) were given to groups each of five clinically healthy adult beagles. Measurements of heparin activity with a factor Xa-dependent chromogenic substrate, activated partial thromboplastin time (APTT) (two different reagents), thrombin time (TT, two different thrombin activities in the %%%reagent%%% : 3 and 6 IU ml-1) and the reaction time of the %%%resonance%%% thrombogram (RTG-r) with two different measuring devices were performed at different times. The relationship between ratio values (actual/baseline values) of the coagulation tests and heparin activity was analysed based on regression analysis and correlation coefficient. The greatest alterations were seen for the TT(3 IU ml-1) and the RTG-r which were near or exceeded the upper limit of measuring range, if 25 IU kg-1 BW heparin were given i.v. at heparin plasma levels of 0.54+-0.13 IU ml-1. These results show, that only APTT and TT measured with high thrombin activity assay appear suitable for guiding high dose heaprin therapy in dogs. Averaged alterations of APTT ratio in canine plasma were less than those observed in people for similar plasma heaprin levels, indicating that the guideline extrapolated from people for monitoring high dose heparin therapy using APTT may not be valid for use in dogs. After coagulation times had been converted into ratio values, based on regression analysis and Wilcoxon's test, differences of heparin sensitivity were found not only for TT measured with different thrombin activities but also for different APTT reagents ($P<0.001$). The correlation between amidylotic antifactor Xa activity and ratio of coagulation times was only moderate and found to be lower for RTG-r

(instrument 1: rs=0.711; instrument 2: rs=0.573) than for the other coagulation tests (rs=0.822 to rs=0.890). This indicates a considerable variability of the ratio values of the screening tests at defined heparin plasma activities. These results show, that blood coagulation tests in general are little or unsuitable for heparin antifactor-Xa activity control.

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16084119 BIOSIS NO.: 200100255958
Antivascular treatment of solid melanoma tumors with bacteriochlorophyll-serine-based photodynamic therapy
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JOURNAL: Photochemistry and Photobiology 73 (3): p257-266 March, 2001 2001
MEDIUM: print
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RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: We describe here a strategy for photodynamic eradication of solid melanoma tumors that is based on photo-induced vascular destruction. The suggested protocol relies on synchronizing illumination with maximal circulating drug concentration in the tumor vasculature attained within the first minute after %%%administrating%%% the sensitizer. This differs from conventional photodynamic therapy (PDT) of tumors where illumination coincides with a maximal concentration differential of sensitizer in favor of the tumor, relative to the normal surrounding tissue. This time window is often achieved after a delay (3-48 h) following sensitizer %%%administration%%%. We used a novel photosensitizer, bacteriochlorophyll-serine (Bchl-Ser), which is water soluble, highly toxic upon illumination in the near-infrared (lambdamax 765-780 nm) and clears from the circulation in less than 24 h. Nude CD1 mice bearing malignant M2R melanotic melanoma xenografts (76-212 mm3) received a single complete treatment session. Massive vascular damage was already apparent 1 h after treatment. Changes in vascular permeability were observed in vivo using contrast-enhanced magnetic %%%resonance%%% imaging (MRI), with the contrast %%%reagent%%% Gd-DTPA, by shortening spin-spin relaxation time because of hemorrhage formation and by determination of vascular macromolecular leakage. Twenty-four hours after treatment a complete arrest of vascular perfusion was observed by Gd-DTPA-enhanced MRI. Histopathology performed at the same time confirmed primary vascular damage with occlusive thrombi, hemorrhage and tumor necrosis. The success rate of cure of over 80% with Bchl-Ser indicates the benefits of the short and effective treatment protocol. Combining the sensitizer %%%administration%%% and illumination steps into one treatment session (30 min) suggests a clear advantage for future PDT of solid tumors.

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15939938 BIOSIS NO.: 200100111777
The correlation between plasma anti-factor Xa activity and haemostatic tests in healthy dogs, following the %%%administration%%% of a low molecular weight heparin
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JOURNAL: Research in Veterinary Science 69 (3): p241-247 December, 2000
2000
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LANGUAGE: English

ABSTRACT: The aim of the study was to examine how activated partial thromboplastin time (APTT, two different reagents), thrombin time (TT, thrombin activity in the %%%reagent%%%: 3 or 6 IU ml-1) and reaction time of the %%%resonance%%% thrombogram (RTG-r) in healthy dogs are influenced by low molecular weight heparin (LMWH). Three different LMWH doses were given subcutaneously or intravenously to groups, each of five healthy dogs. Mean plasma anti-FXa activities of 0.43, 0.88 and 1.86 anti-FXa IU ml-1 were measured 2 min after intravenous injection of 25, 50 or 100 anti-FXa IU kg-1. At this time, a dose-dependent increase of the coagulation times, above the baseline values ($P < 0.05$), was observed for all haemostatic tests. The significant prolongation of coagulation time lasted 10 minutes to 3 hours, and it was dependent on the test employed and LMWH dose. After subcutaneous LMWH injection of 50, 100 and 200 anti-FXa IU kg-1, significant changes of the coagulation time above initial values were limited to the period around the time when maximum anti-FXa activities (0.23, 0.43 or 0.90 anti-FXa IU ml-1) were observed. For the tests which were less affected by the LMWH (APTT, TT(6 IU ml-1)), only small increases (< 4 seconds) were observed even after the highest subcutaneous LMWH dose. The correlation between plasma heparin activity and the relative alteration compared to the initial value (ratio), of the different coagulation tests was only moderate and considerably lower for RTG-r (rs = 0.526) than for the TT (rs = 0.711(6 IU ml-1), rs = 0.780(3 IU ml-1)) and APTT (rs = 0.667 (%%%reagent%%% 1), rs = 0.727(%%%reagent%%% 2)). The low degree of prolongation, which was found particularly for the group tests APTT and TT(6 IU ml-1), reflects the low anti-thrombin activity of LMWH. The results indicate that measurement of anti-FXa activity with chromogenic substrates is the method of choice to control LMWH therapy in dogs, as is the case in humans.

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15638941 BIOSIS NO.: 200000357254
Monitoring the irradiation-induced conformational changes of ovalbumin by using monoclonal antibodies and surface plasmon %%%resonance%%%
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JOURNAL: Bioscience Biotechnology and Biochemistry 64 (4): p710-716 April, 2000 2000
MEDIUM: print
ISSN: 0916-8451
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ABSTRACT: Two types of conformationally specific anti-irradiated ovalbumin monoclonal antibodies were prepared in order to study and monitor irradiation-induced structural changes in the ovalbumin molecule. Surface plasmon %%%resonance%%% (SPR) detection was used to investigate the kinetic parameters of the reaction between antibodies and ovalbumin which had been %%%administered%%% with different doses of irradiation (0, 1.5, 2.0, 5.0, 10, 20, 50, and 100 kGy). The results demonstrate that the combination of monoclonal antibodies and the SPR method can be used to characterize the irradiation-induced conformational change with an unlabelled %%%reagent%%%.

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15321118 BIOSIS NO.: 200000039431
Hemoglobin toxicity in experimental bacterial peritonitis is due to production of reactive oxygen species
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JOURNAL: Clinical and Diagnostic Laboratory Immunology 6 (6): p938-945 Nov., 1999 1999
MEDIUM: print
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LANGUAGE: English

ABSTRACT: Hemoglobin (Hb) is a toxic molecule responsible for the extreme lethality associated with experimental Escherichia coli peritonitis, but the mechanism has yet to be elucidated. Hb, but not globin, showed toxic effects in a live E. coli model but not in a model using killed E. coli. Methamoglobin, hematin, and the well-known Fenton reagents iron and iron-EDTA demonstrated the same lethal effect in E. coliperitonitis as Hb, while the addition of the Fenton inhibitors desferrioxamine (DF) and diethylenetriamine pentaacetate removed most of the cytotoxic activity of iron. %%%Administration%%% of a combined dose of superoxide dismutase and catalase minimized the action of Hb and iron-EDTA, suggesting that both O2.- and H2O2 are involved in the toxic action of Hb in this rat model. The combination of the antioxidative enzymes and DF further suppressed iron-mediated lethality. An electron spin %%%resonance%%% technique with the spin-trapping %%%reagent%%% 5,5-dimethyl-1-pyroline-N-oxide (DMPO) showed O2.- generation in the peritoneal fluid of rats injected with E. coli alone or E. coli plus iron-DF, and .OH generation was detected in

the peritoneal fluid of the rats injected with iron-EDTA. Hb did not show any spin adduct of oxygen radicals, suggesting that Hb produces non-spin-trapping radical ferryl ion, which decayed the spin adduct of DMPO. In the presence of Hb or iron-EDTA, O2--generating activity and viability of phagocytes decreased, whereas lipid peroxidation of peritoneal phagocytes increased. Generation of oxygen radicals and lipid peroxidation did not differ in the live and dead bacterial models. Bacterial numbers in the peritoneal cavity and blood were markedly increased in the live bacterial model with Hb and iron-EDTA. The Fenton inhibitor iron-DF prevented the loss of phagocyte function, lipid peroxidation, and bacterial proliferation. These results led us to conclude that the lethal toxicity of Hb in bacterial peritonitis is associated with a Fenton-type reaction, the products of which decrease phagocyte viability, through the induction of lipid peroxidation, allowing bacterial proliferation and resulting in mortality.

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15124373 BIOSIS NO.: 199900384033
Simultaneous analysis of urinary 2-thiothiazolidine-4-carboxylic acid and thiocarbamide as a biological exposure index for carbon disulfide exposure
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JOURNAL: Yonsei Medical Journal 40 (3): p265-272 June, 1999 1999
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LANGUAGE: English

ABSTRACT: The objectives of this study were to develop optimal analytic methods for detecting urinary 2-thiothiazolidine-4-carboxylic acid (TTCA) and thiocarbamide simultaneously and to evaluate the usefulness of these metabolites to a biological exposure index (BEI) for carbon disulfide (CS2) exposure. For this experiment, synthesized TTCA and thiocarbamide were used. The synthesized TTCA was identified by infrared spectrophotometer, nuclear magnetic %%%resonance%%% spectrometer and thin layer chromatography. The recovery rates of both metabolites were calculated to find the optimum analytical method. The amounts of urinary TTCA and thiocarbamide were measured by using an ultraviolet detector connected to high performance liquid chromatography (HPLC) after the %%%administration%%% of CS2 (350, 700 mg/kg) into Sprague-Dawley rats intraperitoneally. The maximum absorbance wave lengths for TTCA and thiocarbamide were 272 and 236 nm, respectively. Ethyl acetate extraction with NaCl as a salting-out %%%reagent%%% was used as a simultaneous extraction method for these metabolites. HPLC conditions for these metabolites included using a NH2 column, 50 mM KH2PO4: acetonitrile (85 : 15) and pH 3. Excreted amounts of urinary TTCA and thiocarbamide were increased significantly following CS2 %%%administration%%%. TTCA, which was already adopted as a BEI for CS2 by the American Conference of Governmental Industrial Hygienists (ACGIH), seems to be a more useful BEI

for CS2 exposure than thiocarbamide. However further studies are needed to increase analytical efficiency before thiocarbamide can be adopted as a BEI and to apply this analytic method for simultaneous analysis of these metabolites in workers exposed to CS2.

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13695509 BIOSIS NO.: 199799329569
Direct determination of diastereomeric 13C-labeled ketoprofen glucuronides in human urine by nuclear magnetic %%%resonance%%% spectroscopy
AUTHOR: Akira Kazuki (Reprint); Shinohara Yoshihiko
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JOURNAL: Analytica Chimica Acta 334 (1-2): p67-74 1996 1996
ISSN: 0003-2670
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: A new method based on the 13C-labeling and nuclear magnetic %%%resonance%%% (NMR) spectroscopy was developed to study the human urinary excretion of diastereomeric acylglucuronides after the oral %%%administration%%% of 100 mg racemic (3-13C)ketoprofen (KP). The urinary excretion of diastereomeric (3-13C)KP glucuronides formed from (3-13C)KP was followed by proton-decoupled 13C NMR spectroscopy (9.4 T, 10 min accumulation time) without any separation procedures such as extraction and chromatography. The C3 resonances due to the diastereomers were satisfactorily separated from each other by the addition of methyl-beta-cyclodextrin as an achiral shift %%%reagent%%% through the formation of inclusion complexes. The C3 resonances were detected with an acceptable signal-to-noise ratio over 8 h at a therapeutic dosage. The concentrations of (13C)KP glucuronides were calculated from the inverse gated decoupling and proton-decoupling experiments using (2-13C)glycine as an internal standard. The urinary excretion of diastereomeric (3-13C)KP glucuronides over 8 h amounted to 29% (downfield position) and 25% (upfield position) of the %%%administered%%% dose, respectively, and the former was presumed to have S-configuration. The elimination rate constants of both glucuronides were virtually the same (K=0.19 h-1). In addition, the susceptibility of the glucuronides to acyl migration as well as hydrolysis was suggested under the physiological conditions (37 degree C, pH 7.3). The present direct approach is simple and convenient, and has the potential for wide application to the analysis of the labile acylglucuronides of 2-arylpropionic acids in biofluids.

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13511933 BIOSIS NO.: 199699145993
Enhanced NO production during Mg deficiency and its role in mediating red blood cell glutathione loss
AUTHOR: Mak I Tong (Reprint); Komarov Andrei M; Wagner Tammy L; Stafford Richard E; Dickens Benjamin F; Weglicki William B

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JOURNAL: American Journal of Physiology 271 (1 PART 1): pC385-C390 1996 1996
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ABSTRACT: The effect of dietary Mg deficiency on nitric oxide (NO) production and its role in mediating oxidative depletion of red blood cell (RBC) glutathione in rats were investigated. Male Sprague-Dawley rats were placed on Mg-deficient or Mg-sufficient diets for up to 3 wk. Plasma nitrate plus nitrite levels, determined by the Escherichia coli reductase/Griess %%%reagent%%% procedures, increased 1.7-fold during the 1st wk and increased 2- to 2.4-fold during the 2nd and 3rd wk on the Mg-deficient diet. In association, substantial losses (apprx 50%) of RBC glutathione occurred during the 2nd and 3rd wk. %%%Administration%%% of the NO synthesis inhibitor NG-nitro-L-arginine methyl ester (L-NAME) in drinking water (0.5 mg/ml) effectively blunted the increases in plasma nitrate/nitrite during Mg deficiency. Concomitantly, losses of RBC glutathione exhibited by Mg-deficient rats were significantly attenuated. Packed RBCs, obtained from Mg-deficient but not from Mg-sufficient animals, displayed a prominent nitrosyl hemoglobin signal detected by electron spin %%%resonance%%% spectroscopy; the signals of the samples from the L-NAME-treated Mg-deficient rats were greatly reduced. With isolated RBCs, losses of the glutathione could be induced directly by peroxynitrite or 3-morpholinosydnonimine, which generates NO + cntdot O-2-, but not by NO (from sodium nitroprusside) alone, in a concentration-dependent manner. The results clearly indicate that NO overproduction occurs and participates in RBC glutathione loss during Mg deficiency. Because neutrophil activation also occurs, we suggest that NO might interact with superoxide anions to form peroxynitrite, which then directly oxidizes RBC glutathione.

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13036326 BIOSIS NO.: 199598504159
Interference of gadodiamide injection (OMNISCAN) on the colorimetric determination of serum calcium
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JOURNAL: Scandinavian Journal of Clinical and Laboratory Investigation 55 (5): p421-426 1995 1995
ISSN: 0036-5513
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RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The interference of the non-ionic magnetic %%%resonance%%% contrast medium gadodiamide injection (OMNISCAN, Nycomed Imaging, Oslo, Norway) in the colorimetric determination of serum calcium has been investigated in commercial reconstituted serum, and in serum from rabbits

and humans dosed with the contrast medium. Inductively coupled plasma-atomic emission spectroscopy (ICP-AES) and ion-selective electrodes were used as reference methods for analysis of serum calcium. The results showed that the colorimetric %%%reagent%%% kit gave an apparent decrease in serum calcium after %%%administration%%% of a clinical dose of gadodiamide injection, and that the extent of interference is correlated to the concentration of the contrast medium. However, serum calcium was not changed when measured by means of an ion-selective electrode or ICP-AES. It is therefore recommended that colorimetric %%%reagent%%% kits should not be used for determination of serum calcium in samples taken within the first 24 h after %%%administration%%% of gadodiamide injection.

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12681205 BIOSIS NO.: 199598149038
Apparent inhibition of superoxide dismutase activity in vitro by diesel exhaust particles
AUTHOR: Kumagai Yoshito (Reprint); Taira Junsei; Sagai Masaru
AUTHOR ADDRESS: Res. Team Health Effects Air Pollutants, Natl. Inst. Environ. Studies, 16-2 Onogawa, Tsukuba, Ibaraki 305, Japan**Japan
JOURNAL: Free Radical Biology and Medicine 18 (2): p365-371 1995 1995
ISSN: 0891-5849
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The inhibitory effects of diesel exhaust particles (DEP) on superoxide dismutase (SOD) activity were examined in vitro because intratracheal %%%administration%%% of DEP to mice resulted in a suppression of the pulmonary enzyme activity (Sagai et al., Free Radic. Biol. Med. 14:37-47; 1993). Superoxide production, based on the reduction of cytochrome c, was suppressed considerably by the soluble fraction of mouse lung and by purified SOD from bovine erythrocytes, but the suppression was drastically diminished in the presence of methanol-extractable compounds of DEP. Inhibition of SOD by diethyldithiocarbamate was irreversible, but that by 1,2-naphthoquinone (1,2-NQ) and the methanol extract of DEP was removed by dialysis. Inhibition of superoxide mediated cytochrome c reduction by Tiron, a scavenging agent for superoxide, was blocked by the methanol extract and 1,2-NQ in a concentration-dependent manner. In contrast, addition of a large amount of SOD to the reaction mixture resulted in an almost complete disappearance of inhibitory action of not only 1,2-NQ but also the methanol extract. The existence of carbonyl compounds in the DEP was confirmed by thin-layer chromatography (TLC) with 2,4-dinitrophenylhydrazine %%%reagent%%%. Electron spin %%%resonance%%% (ESR) spectra of an incubation mixture of oxidized 1,2-dihydroxynaphthalene in the absence and presence of cytochrome c indicated a reaction between the semiquinone radical of 1,2-NQ and cytochrome c. These results indicate that the apparent reduction in SOD activity by DEP is due to the chemical reaction of superoxide with components like quinones, which reduce levels of superoxide.

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12405181 BIOSIS NO.: 199497426466
Electron spin %%%resonance%%% spin-trapping investigation into the effects of paraquat and desferrioxamine on hydroxyl radical generation during acute iron poisoning
AUTHOR: Burkitt Mark J (Reprint); Kadiiska Maria B; Hanna Phillip M; Jordan Sandra J; Mason Ronald P
AUTHOR ADDRESS: Rowett Res. Inst., Greenburn, Rd., Bucksburn, Aberdeen, AB2 9SB, Scotland, UK**UK
JOURNAL: Molecular Pharmacology 43 (2): p257-263 1993 1993
ISSN: 0026-895X
DOCUMENT TYPE: Article
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LANGUAGE: English

ABSTRACT: We have previously described a secondary radical-trapping technique for the detection of in vivo hydroxyl radical generation during acute iron overload. With this technique, the hydroxyl radical (cntdot OH) reacts with dimethylsulfoxide to form the methyl radical (cntdot CH-3), which is then detected by ESR spectroscopy as its adduct with the spin trap phenyl-N-tert-butylnitrone in the bile of treated animals. In this study, we report both the individual and combined effects of the futile-cycling agent paraquat (PQ-2+) and the iron-chelating agent desferrioxamine (DFO) on iron-dependent cntdot OH generation. Interactions between iron and the partially reduced oxygen species superoxide and hydrogen peroxide, which are generated during the metabolism of PQ-2+, might be expected to stimulate cntdot OH generation to a level above that seen in the presence of the metal ion alone. Although PQ-2+ was often found to promote further cntdot OH generation when %%%administered%%% to animals also given iron, the large variation observed between individual animals in response to the %%%reagent%%% meant that the effect was not statistically significant (p lt 0.05). DFO was found to abolish iron-de pendent cntdot OH generation, both in the presence and in the absence of PQ-2+. This is believed to result from the chelation of iron by DFO, to form an essentially redox-inert iron(III) complex that is unable to catalyze cntdot OH radical formation. In addition, it was found that the iron(II) complex of DFO can reduce PQ-2+ to its radical cation in vitro, indicating, therefore, that the chelation of iron by DFO may not necessarily prevent its participation in free radical reactions.

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11389435 BIOSIS NO.: 199294091276
INHIBITION OF RADICAL ADDUCT REDUCTION AND REOXIDATION OF THE CORRESPONDING HYDROXYLAMINES IN IN-VIVO SPIN TRAPPING OF CARBON TETRACHLORIDE-DERIVED RADICALS
AUTHOR: SENTJURC M (Reprint); MASON R P
AUTHOR ADDRESS: J STEFAN INST, JAMOVA 39, 61000, LJUBLJANA, SLOVENIA** SLOVENIA
JOURNAL: Free Radical Biology and Medicine 13 (2): p151-160 1992

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RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: In vivo spintrapping of radical metabolites has become a promising tool in understnding and predicting toxicities caused by different xenobiotics. However, in biological systems radical adducts can be reduced to electron paramagnetic %%%resonance%%% (EPR)-silent hydroxylamines. To overcome this difficulty, different procedures for reoxidation of the reduced radical adducts were systematically investigated and some metabolic inhibitors of nitroxide reduction were tested. As a test system, carbontetrachloride (CCl4), a known hepatotoxic substance, was used. CCl4 is metabolized by liver to .CCl3 and, in the presence of the spin trap phenyl N-t-butylnitrone (PBN), forms the PBN/.CCl3 and PBN/.CO2- radical adducts. These radicals adducts were measured in the bile using electron paramagnetic %%%resonance%%% after %%%administration%%% of CCl4 and PBN to the rat. We have shown that these radical adducts were reduced to the corresponding hydroxylamines in vivo, since immediately after the collection of bile only traces of the radicals adduct could be detected, but after oxidation by different procedures such as bubbling with oxygen, addition of mild oxidant potassium ferricyanide or autoxidation the EPR spectra intensity increases, indicating that the hydroxylamines had been re-oxidized back to nitroxides. The collection of bile into plastic Eppendorf tubes containing the sulfhydryl %%%reagent%%% N-ethylmaleimide (NEM) or the enzyme ascorbate oxidase did not increase the intensity of the spectra significantly, demonstrating that neither reduction by reduced glutathione (GHS) nor ascorbic acid occurred ex vivo. However in the presence of NEM faster re-oxidation was observed. A new radical adduct that was not observed previously in any in vivo experiment and which exhibited 13C hyperfine coupling was detected when the rats were injected with 13CCl4. We have proven that this is the same adduct detected previously in vitro in microsomal incubations of CCl4, PBN, GSH, and reduced nicotinamide adenine dinucleotide phosphate (NADPH). As a general rule, we have shown that a variety of oxidation procedures should be tried to detect the different radical adducts which are otherwise not observable due to the in vivo reduction of radical adducts.

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10839541 BIOSIS NO.: 199192085312
MONITORING OF INTRACELLULAR AMMONIUM IN PERFUSED RAT SALIVARY GLAND BY NITROGEN-14 NMR SPECTROSCOPY
AUTHOR: SEO Y (Reprint); MURAKAMI M
AUTHOR ADDRESS: DEP MOL PHYSIOL, NATL INST PHYSIOLOGICAL SCI, OKAZAKI 444, JPN**JAPAN
JOURNAL: Proceedings of the Royal Society of London Series B Biological Sciences 244 (1311): p191-196 1991
ISSN: 0080-4649
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LANGUAGE: ENGLISH

ABSTRACT: We have observed the changes in the intracellular ammonium (NH4+) content and the intracellular pH during %%%administration%%% of 20 mM NH4Cl (the ammonium pulse experiment) using nitrogen-14 and phosphorus-31 nuclear magnetic %%%resonance%%% spectroscopy (14N and 31P NMR) at 8.45 T. In the isolated perfused rat mandibular salivary gland, resonances of trimethylamines (-328 p.p.m.) and betaine (-329 p.m.m. from the %%%resonance%%% of NO3-) were detected. A chemical shift %%%reagent%%%, 10 mM of dysprosium triethylenetetramine-N,N,N',N'',N''',N'''-hexaacetic acid (Dy(TTHA)), was used to discriminate between the resonances from the extracellular (NH4+ (-352 p.p.m.) and the intracellular NH4+ (-355 p.p.m.). During the NH4Cl application, the intracellular NH4+ content ([NH4+]i) increased quickly to ca. 50 mmol per litre intracellular fluid (ICF), then increased gradually to ca. 70 mmol per litre ICF. The intracellular pH (pHi), calculated from the 31P chemical shift of inorganic phosphate, increased transiently by 0.5 pH units and then decreased gradually in spite of the high level of [NH4+]i. The initial increase of [NH4+]i, which was observed by 14N NMR, was larger than that calculated from the intracelluar pH on an assumption of a non-ionic diffusion process for ammonia. These results suggest a possibility of influx of NH4+, and also suggest an activation of cellular buffering mechanism that extrudes the excess bases from the cells.

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09605699 BIOSIS NO.: 198987053590
DEXTRAN-MAGNETITE A CONTRAST AGENT FOR SODIUM-23 MRI
AUTHOR: SUMMERS R M (Reprint); JOSEPH P M; RENSHAW P F; KUNDEL H L
AUTHOR ADDRESS: PENDERGRASS DIAGNOSTIC RADIOL RES LAB, DEP RADIOL, UNIV PA SCH MED, PHILADELPHIA, PA 19104, USA**USA
JOURNAL: Magnetic Resonance in Medicine 8 (4): p427-439 1988
ISSN: 0740-3194
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: Dextran-magnetite, a superparamagnetic compound, is a powerful relaxation %%%reagent%%% for sodium. %%%Administered%%% intravenously, it is confined mainly to the vasculature and eliminates the signal from plasma sodium, a significant component of the tissue sodium signal. Applications of dextran-magnetite for in vivo sodium imaging of a normal rat and rats with a tumor and experimentally induced peripheral edema are shown. Our results indicate that dextran-magnetite may be useful for improving tumor detection and for imaging of edema. To our knowledge, this is the first report of a contrast agent suitable for sodium magnetic %%%resonance%%% imaging.

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19043780 BIOSIS NO.: 200600389175

Purification and characterization of a high specificity polyclonal antibody to the adenomatous polyposis coli tumour suppressor protein
AUTHOR: Catimel Bruno; Nice Edouard C (Reprint); Karrlander Maria; Ross Janine; Catimel Jenny; Burgess Antony W; Faux Maree
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JOURNAL: Biomedical Chromatography 20 (6-7): p569-575 JUN-JUL 2006 2006
ISSN: 0269-3879
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Recombinant proteins, commonly expressed in %%%fusion%%% with an affinity tag to facilitate purification, are often used as immunogens for polyclonal antibody production. Careful immunopurification of the antibody product is often the key to obtaining a high-specificity polyclonal antibody against the protein domain of interest. This study describes the purification and characterization of such an antibody directed against the adenomatous polyposis coli (APC) tumour suppressor. We used a combination of affinity chromatography and biosensor analysis to optimize and monitor antibody purification. This antibody was then characterized by immunoprecipitation, proteomic analyses and immunofluorescence staining and shown to be a valuable %%%reagent%%% for the study of APC biology. Using this antibody we successfully isolated and identified APC, using MS/MS, from transfected cell lines. A novel phosphorylation site on APC was identified at ser 1436. Similar strategies involving multiple immuno-affinity steps coupled with surface plasmon %%%resonance%%% (SPR), immunoprecipitation proteomic and immunofluorescence analyses should be generally applicable for the purification and characterization of other polyclonal antibodies.
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18520538 BIOSIS NO.: 200510215038
Time-resolved Forster %%%resonance%%% energy transfer assays for the binding of nucleotide and protein substrates to p38 alpha protein kinase
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JOURNAL: Analytical Biochemistry 343 (1): p76-83 AUG 1 2005 2005
ISSN: 0003-2697
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: We have developed assays for the binding of nucleotide and protein substrates to p38 alpha protein kinase based on time-resolved Forster %%%resonance%%% energy transfer. p38 alpha was biotinylated by addition of a sequence that targets biotin to a single lysine when

co-expressed with biotin ligase in Escherichia coli, allowing formation of a complex between a streptavidin "LANCE" europium chelate conjugate and p38a. When this %%%reagent%%% was combined with M39AF, a p38 inhibitor containing a fluorescent moiety whose excitation wavelengths match the emission wavelengths of the europium chelate, a change in ratio of light emitted at 665 nm/615 nm is detected. Less than 100 pM complex was detected with a signal/background ratio of > 30-fold. The complex exhibits slow, tight binding kinetics where the apparent K-d decreases with a relaxation time of 21 min at 125 pM biotin p38 alpha. Preincubating inhibitors or ATP with biotin-pMot and adding M39AF as a competitor yielded IC(50)s consistent with those measured by enzyme assay for the activated form of biotin-pH alpha. The same technique was also used to measure affinity of inhibitors for the unphosphorylated and catalytically inactive form of biotin-pMot. To measure affinity of p38 alpha for its protein substrate MK2, we incubated biotin-p38 alpha with a glutathione S-transferase MK2 %%%fusion%%% protein. Detection of the complex after incubation with streptavidin-allophycocyanin and a LANCE-conjugated anti-GST allowed measurement of affinity of MK2 for biotin-p38 alpha and detection of 0.5 nM p38 alpha center dot MK2 complex with signal/background ratio > 5-fold. Competition with unbiotinylated p38 alpha yielded an IC50 value of 5 nM. Activation of either p38 alpha or MK2 had no effect on the measured K-d. M39AF was found to bind in a ternary complex with p38 alpha - MK2 with lower affinity than that observed in the binary complex with p38 alpha alone. (c) 2005 Elsevier Inc. All rights reserved.

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17808125 BIOSIS NO.: 200400178882
Perfusion of 99Tcm-labeled CD105 Mab into kidneys from patients with renal carcinoma suggests that CD105 is a promising vascular target.
AUTHOR: Costello Brendan; Li Chenggang; Duff Sarah; Butterworth David; Khan Ali; Perkins Michael; Owens Susan; Al-Mowallad Abdul Fattah; O'Dwyer Sarah; Kumar Shant (Reprint)
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JOURNAL: International Journal of Cancer 109 (3): p436-441 10 April, 2004 2004
MEDIUM: print
ISSN: 0020-7136
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: There is strong published and unpublished evidence that our CD105 Mab E9, which is highly reactive with angiogenic endothelial cells, could be a useful %%%reagent%%% to target the vasculature of solid tumors in man. Since Mab E9 does not cross-react with animal tissues, we undertook here to evaluate its localization using human kidney as an ex vivo model. Perfusion was performed through the renal artery of 99Tcm-labeled purified CD105 Mab in freshly excised kidneys from 7 patients with renal carcinoma. In all 7 cases, immunoscintigraphs showed the presence of well-defined radioactive hot spots, which matched the positions of the

tumors as identified by presurgery MRI scans and subsequent histopathologic examination. Importantly, in one instance, where a presurgery MRI scan had identified only one tumor, immunoscintigraphs showed 2 distinct hot spots of radioactivity. The pathology report confirmed that the additional hot spot corresponded to a small secondary well-vascularized tumor. The implication of this finding is that the radiolabeled Mab, E9, may be of use in the detection of metastatic disease. That the labeling of tumors was specific was confirmed when prior perfusion of unlabeled mab E9 in 2 kidneys completely blocked the localization of 99Tcm-conjugated Mab E9. Radioactivity in samples of tumor and normal tissue taken from 7 kidneys was counted in a gamma counter. In all cases, there was a greater uptake of radioactivity in tumors compared with the corresponding normal kidneys. The median values, adjusted per gram wet weight, for 99Tcm were 14.8 times (range, 4.8-113.0) greater in kidney tumors than in normal kidney tissue ($p < 0.007$). Immunofluorescent staining of cryostat sections of tumor tissues in each of the 7 cases showed strong and uniform localization of Mab E9 in tumor microvessels. Interestingly, %%%chimeric%%% staining of endothelial cells (ECs) was seen in an occasional microvessel segment. That is, while most of the ECs lining a microvessel were strongly stained, an occasional EC was negative. This was not an artifact of staining. Unstained ECs may be nonangiogenic or apoptotic since CD105 is a proliferation/activation-associated antigen. Further investigations are warranted to establish the pharmacokinetics of 99Tcm-labeled CD105 antibody in vivo. This would enable us to determine whether an apparently highly successful ex vivo study has the potential for tumor imaging/therapeutic vascular targeting in patients with cancer.

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16243526 BIOSIS NO.: 200100415365
Optimal inhibition of X4 HIV isolates by the CXC chemokine stromal cell-derived factor 1alpha requires interaction with cell surface heparan sulfate proteoglycans
AUTHOR: Valenzuela-Fernandez Agustin; Palanche Tania; Amara Ali; Magerus Aude; Altmeyer Ralf; Delaunay Thierry; Virelizier Jean-Louis; Baleux Francoise; Galzi Jean-Luc; Arenzana-Seisdedos Fernando (Reprint)
AUTHOR ADDRESS: Unite d'Immunologie Virale and Unite de Chimie Organique, Institut Pasteur, 28 Rue du Dr. Roux, 75724, Paris Cedex 15, France** France
JOURNAL: Journal of Biological Chemistry 276 (28): p26550-26558 July 13, 2001 2001
MEDIUM: print
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The chemokine stromal cell-derived factor 1 (SDF-1) is the natural ligand for CXC chemokine receptor 4 (CXCR4). SDF-1 inhibits infection of CD4+ cells by X4 (CXCR4-dependent) human immunodeficiency virus (HIV) strains. We previously showed that SDF-1alpha interacts specifically with heparin or heparan sulfates (HSs). Herein, we delimited the boundaries of the HS-binding domain located in the first beta-strand

of SDF-1alpha as the critical residues. We also provide evidence that binding to cell surface heparan sulfate proteoglycans (HSPGs) determines the capacity of SDF-1alpha to prevent the fusogenic activity of HIV-1 X4 isolates in leukocytes. Indeed, SDF-1alpha mutants lacking the capacity to interact with HSPGs showed a substantially reduced capacity to prevent cell-to-cell %%%fusion%%% mediated by X4 HIV envelope glycoproteins. Moreover, the enzymatic removal of cell surface HS diminishes the HIV-inhibitory capacity of the chemokine to the levels shown by the HS-binding-disabled mutant counterparts. The mechanisms underlying the optimal HIV-inhibitory activity of SDF-1alpha when attached to HSPGs were investigated. Combining fluorescence %%%resonance%%% energy transfer and laser confocal microscopy, we demonstrate the concomitant binding of SDF-1alpha to CXCR4 and HSPGs at the cell membrane. Using FRET between a Texas Red-labeled SDF-1alpha and an enhanced green fluorescent protein-tagged CXCR4, we show that binding of SDF-1alpha to cell surface HSPGs modifies neither the kinetics of occupancy nor activation in real time of CXCR4 by the chemokine. Moreover, attachment to HSPGs does not modify the potency of the chemokine to promote internalization of CXCR4. Attachment to cellular HSPGs may co-operate in the optimal anti-HIV activity of SDF-1alpha by increasing the local concentration of the chemokine in the surrounding environment of CXCR4, thus facilitating sustained occupancy and down-regulation of the HIV coreceptor.

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15948277 BIOSIS NO.: 200100120116
Demonstration of a homogeneous noncompetitive immunoassay based on bioluminescence %%%resonance%%% energy transfer
AUTHOR: Arai Ryoichi; Nakagawa Hideyuki; Tsumoto Kouhei; Mahoney Walt; Kumagai Izumi; Ueda Hiroshi (Reprint); Nagamune Teruyuki (Reprint)
AUTHOR ADDRESS: Department of Chemistry and Biotechnology, Graduate School of Engineering, University of Tokyo, Hongo, Bunkyo-ku, Tokyo, 113-8656, Japan**Japan
JOURNAL: Analytical Biochemistry 289 (1): p77-81 February 1, 2001 2001
MEDIUM: print
ISSN: 0003-2697
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RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: We describe a noncompetitive homogeneous bioluminescent immunoassay based on the antigen-dependent reassociation of antibody variable domains (open sandwich bioluminescent immunoassay, OS-BLIA). The reassociation of two %%%chimeric%%% proteins, an antibody heavy-chain fragment (VH)-Renilla luciferase (Rluc) and an antibody light-chain fragment (VL)-enhanced yellow fluorescent protein (EYFP), was monitored by a bioluminescence %%%resonance%%% energy transfer (BRET) between the two. Upon simple mixing of the reagents with the sample, an antigen-dependent increase in BRET was observed with a measurable concentration range of 0.1apprx10 mug/ml antigen hen egg lysozyme. Compared with our comparable assays based on fluorescence %%%resonance%%% energy transfer (FRET), a 10-fold improvement in the sensitivity was attained, probably due to a reduction in %%%reagent%%% concentration.

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15722168 BIOSIS NO.: 200000440481
CD47 is a ligand for rat macrophage membrane signal regulatory protein SIRP (OX41) and human SIRPalpha1
AUTHOR: Vernon-Wilson Elizabeth F; Kee Wai-Jing; Willis Antony C; Barclay A Neil; Simmons David L; Brown Marion H (Reprint)
AUTHOR ADDRESS: Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford, OX1 3RE, UK**UK
JOURNAL: European Journal of Immunology 30 (8): p2130-2137 August, 2000 2000
MEDIUM: print
ISSN: 0014-2980
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The rat OX41 antigen is a cell surface protein containing three immunoglobulin superfamily domains and intracellular immunoreceptor tyrosine-based inhibitory motifs (ITIM). It is a homologue of the human signal-regulatory protein (SIRP) also known as SHPS-1, BIT or MFR. Cell activation-induced phosphorylation of the intracellular ITIM motifs induces association with the tyrosine phosphatases SHP-1 and SHP-2. To identify the physiological OX41 ligand, recombinant OX41-CD4d3+4 %%%fusion%%% protein was coupled to fluorescent beads to produce a multivalent cell binding %%%reagent%%%. The OX41-CD4d3+4 beads bound to thymocytes and concanavalin A-stimulated splenocytes. This interaction was blocked by the monoclonal antibody (mAb) OX101. Affinity chromatography with OX101 mAb and peptide sequencing revealed the rat SIRP ligand to be CD47 (integrin-associated protein). A direct interaction between human SIRP and human CD47 was demonstrated using purified recombinant proteins and surface plasmon %%%resonance%%% ruling out the involvement of other proteins known to be associated with CD47. The affinity of the SIRP/CD47 interaction was Kd apprxeq 8 muM at 37degreeC with a koffgtoreq2.1 s-1. The membrane-distal SIRP V-like domain was sufficient for binding to CD47.

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15535113 BIOSIS NO.: 200000253426
15N NMR study of the ionization properties of the influenza virus %%%fusion%%% peptide in zwitterionic phospholipid dispersions
AUTHOR: Zhou Zhe; Macosko Jed C; Hughes Donald W; Sayer Brian G; Hawes John ; Epand Richard M (Reprint)
AUTHOR ADDRESS: Department of Biochemistry, McMaster University Health Sciences Center, 1200 Main St. West, Rm. 4H26, Hamilton, ON, L8N 3Z5, Canada**Canada
JOURNAL: Biophysical Journal 78 (5): p2418-2425 May, 2000 2000
MEDIUM: print
ISSN: 0006-3495
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LANGUAGE: English

ABSTRACT: Influenza virus hemagglutinin (HA)-mediated membrane %%%fusion%%% involves insertion into target membranes of a stretch of amino acids located at the N-terminus of the HA2 subunit of HA at low pH. The pKa of the alpha-amino group of 1Gly of the %%%fusion%%% peptide was measured using 15N NMR. The pKa of this group was found to be 8.69 in the presence of DOPC (1,2-dioleoyl-sn-glycero-3-phosphocholine). The high value of this pKa is indicative of stabilization of the protonated form of the amine group through noncovalent interactions. The shift %%%reagent%%% Pr3+ had large effects on the 15N %%%resonance%%% from the alpha-amino group of Gly1 of the %%%fusion%%% peptide in DOPC vesicles, indicating that the terminal amino group was exposed to the bulk solvent, even at low pH. Furthermore, electron paramagnetic %%%resonance%%% studies on the %%%fusion%%% peptide region of spin-labeled derivatives of a larger HA construct are consistent with the N-terminus of this peptide being at the depth of the phosphate headgroups. We conclude that at both neutral and acidic pH, the N-terminal of the %%%fusion%%% peptide is close to the aqueous phase and is protonated. Thus neither a change in the state of ionization nor a significant increase in membrane insertion of this group is associated with increased fusogenicity at low pH.

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15436465 BIOSIS NO.: 200000154778
Marking synaptic activity in dendritic spines with a calpain substrate exhibiting fluorescence %%%resonance%%% energy transfer
AUTHOR: Vanderklish Peter W; Krushel Leslie A; Holst Brent H; Gally Joseph A; Crossin Kathryn L; Edelman Gerald M (Reprint)
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JOURNAL: Proceedings of the National Academy of Sciences of the United States of America 97 (5): p2253-2258 Feb. 29, 2000 2000
MEDIUM: print
ISSN: 0027-8424
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Excitatory synaptic activity can evoke transient and substantial elevations of postsynaptic calcium. Downstream effects of elevated calcium include the activation of the calcium-dependent protease calpain. We have developed a %%%reagent%%% that identifies dendritic spines in which calpain has been activated. A %%%fusion%%% protein was expressed that contained enhanced yellow and enhanced cyan fluorescent protein (EYFP and ECFP, respectively) linked by a peptide that included the mu-calpain cleavage site from alpha-spectrin. A PDZ-binding site fused to ECFP anchored this protein to postsynaptic densities. The %%%fusion%%% protein exhibited fluorescence %%%resonance%%% energy transfer (FRET), and diminution of FRET by proteolysis was used to localize calpain activity in situ by fluorescence microscopy. Incubation of the %%%fusion%%% protein with calpain in the presence of calcium resulted in the separation of EYFP and ECFP into monomeric fluorophores. In

transiently transfected cell lines and dissociated hippocampal neurons, FRET was diminished by raising intracellular calcium levels with an ionophore or with glutamatergic agonists. Calpain inhibitors blocked these changes. Under control conditions, FRET levels in different dendritic spines of cultured neurons and in hippocampal slices were heterogeneous but showed robust decreases upon treatment with glutamatergic agonists. Immunostaining of cultured neurons with antibodies to a spectrin epitope produced by calpain-mediated digestion revealed an inverse correlation between the amount of FRET present at postsynaptic elements and the concentration of spectrin breakdown products. These results suggest that the FRET methodology identifies sites of synaptically induced calpain activity and that it may be useful in analyzing synapses undergoing changes in efficacy.

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15369849 BIOSIS NO.: 200000088162
Characterization of heparin binding of human extracellular superoxide dismutase
AUTHOR: Lookene Aivar; Stenlund Peter; Tibell Lena A E (Reprint)
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JOURNAL: Biochemistry 39 (1): p230-236 Jan. 11, 2000 2000
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LANGUAGE: English

ABSTRACT: The C-terminal domain of human extracellular superoxide dismutase (hEC-SOD) plays a crucial role in the protein's interaction with heparin. Here we investigated this interaction in more detail by comparing the heparin-binding characteristics of two variants of hEC-SOD: the two %%%fusion%%% proteins containing the hEC-SOD C-terminal domain and a synthetic peptide homologous to the C-terminal. The interaction studies were performed using a surface plasmon %%%resonance%%% based technique on a BIAcore system. It should be emphasized that this is a model system. However, the kinetic constants, as measured, are valid in a comparative sense. Comparison of affinities for size-fractionated heparins revealed that octa- or decasaccharides are the smallest heparin fragments that can efficiently interact with the C-terminal domain of hEC-SOD. At physiological salt concentration, and pH 7.4, the hEC-SOD/heparin interaction was found to be of a high-affinity type, with an equilibrium dissociation constant, Kd, of 0.12 muM, which is 700 and 10-20 times lower than the Kd values for the synthetic peptide and the %%%fusion%%% proteins, respectively. However, when an alpha-helical structure was induced in the synthetic peptide, by addition of 10% trifluoroethanol, the Kd decreased to 0.64 muM. The differences in the Kd values were mainly governed by differences in the association rate constants (kappaass). The hEC-SOD/heparin interaction itself was found to have a fairly high dissociation rate constant (0.1 s-1), and a very high association rate constant (8 X 105 M-1 s-1), suggesting that the interaction is mainly controlled by the association. These results together with circular dichroism spectra of the synthetic peptide suggest

that an alpha-helical structure in the C-terminal is essential for optimal binding to heparin and that other parts of hEC-SOD moderate the affinity. Our data also demonstrate that the tetramerization itself does not substantially increase the affinity.

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15319719 BIOSIS NO.: 200000038032
A multi-step lipid mixing assay to model structural changes in cationic lipoplexes used for in vitro transfection
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JOURNAL: Biochimica et Biophysica Acta 1461 (1): p27-46 Nov. 9, 1999 1999
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LANGUAGE: English

ABSTRACT: Formation of liposome/polynucleotide complexes (lipoplexes) involves electrostatic interactions, which induce changes in liposome structure. The ability of these complexes to transfer DNA into cells is dependent on the physicochemical attributes of the complexes, therefore characterization of binding-induced changes in liposomes is critical for the development of lipid-based DNA delivery systems. To clarify the apparent lack of correlation between membrane %%%fusion%%% and in vitro transfection previously observed, we performed a multi-step lipid mixing assay to model the sequential steps involved in transfection. The roles of anion charge density, charge ratio and presence of salt on lipid mixing and liposome aggregation were investigated. The %%%resonance%%% -energy transfer method was used to monitor lipid mixing as cationic liposomes (DODAC/DOPE and DODAC/DOPC; 1:1 mole ratio) were combined with plasmid, oligonucleotides or Na2HPO4. Cryo-transmission electron microscopy was performed to assess morphology. As plasmid or oligonucleotide concentration increased, lipid mixing and aggregation increased, but with Na2HPO4 only aggregation occurred. NaCl (150 mM) reduced the extent of lipid mixing. Transfection studies suggest that the presence of salt during complexation had minimal effects on in vitro transfection. These data give new information about the effects of polynucleotide binding to cationic liposomes, illustrating the complicated nature of anion induced changes in liposome morphology and membrane behavior.

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15102883 BIOSIS NO.: 199900362543
A high throughput assay of the hepatitis C virus nonstructural protein 3 serine proteinase
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JOURNAL: Journal of Virological Methods 80 (1): p77-84 June, 1999 1999
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ABSTRACT: A simple assay was developed based on intramolecular fluorescence %%%resonance%%% energy transfer for detection of the activity of hepatitis C virus (HCV) serine proteinase. Two quenched-flourogenic substrates, (7-methoxycoumarin-4-yl)acetyl (Mca) Asp-Asp-Ile-Val-Pro-Cys-Ser-Met-Ser-(2,4-dinitrophenyl, Dnp) Lys (Mca-Asp-Asp-Ile-Val-Pro-Cys-Ser-Met-Ser-Lys(Dnp), QF-1) and Mca-Asp-Asp-Ile-Val-Pro-Cys-Ser-Met-Lys(Dnp)-Arg-Arg (QF-2), which derived from the NS5A/5B junction of the HCV polyprotein, were designed. Kinetic studies revealed that QF-1 and QF-2 had high affinity for a recombinant enzyme which is a %%%fusion%%% protein of maltose binding protein and almost entire nonstructural protein (MBP-NS3), with Km values comparable to that of longer substrate based on the same cleavage site. QF-1 and QF-2 were cleaved by MBP-NS3 efficiently with kcat values of 7.5 and 4.2 min-1, respectively. QF-2 was also found to be a good substrate of DELTANS3 which contained serine proteinase part of NS3 with kcat value of 4.3 min-1. The cleavage reaction is detected continuously by the elevation of the fluorescence due to release from quenching. The fluorescence of the substrates increases in proportion to progress of the cleavage reaction under the standard conditions. This method was applied for screening of HCV serine protease inhibitors using a fluorescence multiwell plate reader. A group of natural occurring products, flavonoids, was subjected to be screened. Two flavonoids out of 25 were found to inhibit the enzyme moderately at a concentration of 100 muM. The data agreed with those obtained by high-performance liquid chromatography (HPLC). This method is suited to sensitive quantitation of the enzyme reaction as well as the high throughput analysis of the inhibitors.
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19413337 BIOSIS NO.: 200700073078
CD4 and CCR5 constitutively interact at the plasma membrane of living cells - A confocal fluorescence %%%resonance%%% energy transfer-based approach
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JOURNAL: Journal of Biological Chemistry 281 (49): p37921-37929 DEC 8 2006 2006

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LANGUAGE: English

ABSTRACT: Human immunodeficiency virus entry into target cells requires sequential interactions of the viral glycoprotein envelope gp120 with CD4 and chemokine receptors CCR5 or CXCR4. CD4 interaction with the chemokine receptor is suggested to play a critical role in this process but to what extent such a mechanism takes place at the surface of target cells remains elusive. To address this issue, we used a confocal microspectrofluorimetric approach to monitor fluorescence %%%resonance%%% energy transfer at the cell plasma membrane between enhanced %%%blue%%% and green fluorescent proteins fused to CD4 and CCR5 receptors. We developed an efficient fluorescence %%%resonance%%% energy transfer analysis from experiments carried out on individual cells, revealing that receptors constitutively interact at the plasma membrane. Binding of R5-tropic HIV gp120 stabilizes these associations thus highlighting that ternary complexes between CD4, gp120, and CCR5 occur before the %%%fusion%%% process starts. Furthermore, the ability of CD4 truncated mutants and CCR5 ligands to prévent association of CD4 with CCR5 reveals that this interaction notably engages extracellular parts of receptors. Finally, we provide evidence that this interaction takes place outside raft domains of the plasma membrane.

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19344632 BIOSIS NO.: 200700004373
Discriminating in vitro cell %%%fusion%%% from cell aggregation by flow cytometry combined with fluorescence %%%resonance%%% energy transfer
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JOURNAL: Journal of Virological Methods 138 (1-2): p17-23 DEC 2006 2006
ISSN: 0166-0934
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LANGUAGE: English

ABSTRACT: Expression of %%%fusion%%% proteins in the plasma membrane enables cells to bind and fuse with surrounding cells to form syncytia. Cell %%%fusion%%% can have important functional outcomes for the interacting cells, as syncytia formation does in AIDS pathogenesis. Studies on cell %%%fusion%%% would be facilitated by a quantitative method able to discriminate between cellular aggregates and bona fide fused cells in a cell population. Flow cytometry with fluorescence %%%resonance%%% energy transfer is applied here for analyzing %%%fusion%%% of HIV-1 envelope-expressing cells with CD4(+) Jurkat cells. %%%Fusion%%% partners were labeled with the vital lipophilic fluorescent probes DiO (green) and DiI (%%%red%%%) and FRET is manifested by an enhancement of the DiI %%%red%%% fluorescence intensity in double

fluorescent cells, thus allowing discrimination between fused and aggregated cells. The inhibitory effect of anti-CD4 monoclonal antibodies and the inhibitory peptide T-20 upon cell %%%fusion%%% were readily quantified by this technique. This method allows the distinction of fused and aggregated cells even when they are at low frequencies. (c) 2006 Elsevier B.V. All rights reserved.

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19241741 BIOSIS NO.: 200600587136
%%%Resonance%%% energy transfer between green fluorescent protein variants: Complexities revealed with myosin %%%fusion%%% proteins
AUTHOR: Zeng Wei; Seward Harriet E; Malnasi-Csizmadia Andras; Wakelin Stuart; Woolley Robert J; Cheema Gurpreet S; Basran Jaswir; Patel Trushar R; Rowe Arthur J; Bagshaw Clive R (Reprint)
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JOURNAL: Biochemistry 45 (35): p10482-10491 SEP 5 2006 2006
ISSN: 0006-2960
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LANGUAGE: English

ABSTRACT: Green fluorescent protein and its variants are frequently used as Forster (fluorescence) %%%resonance%%% energy transfer (FRET) pairs to determine the proximity of protein domains. We prepared %%%fusion%%% proteins comprising yellow fluorescent protein- Dictyostelium myosin II motor domain-cyan fluorescent protein (YFP-myosin-CFP) and compared their FRET properties with an existing construct (GFP-myosin-BFP), containing a green fluorescent protein acceptor and %%%blue%%% fluorescent protein donor [Suzuki, Y., Yasunaga, T., Ohkura, R., Wakabayashi, T. and Sutoh, K. (1998) Nature 396, 380-383]. The latter construct showed an apparent 40% reduction in acceptor fluorescence on ATP addition, when excited via the donor, compared with the YFP-myosin-CFP constructs which showed a small increase (<= 5%). We propose that this disparity primarily arises from the differential response of GFP and YFP on intramolecular association with the donor probe. Studies with isolated GFP and YFP at high concentrations show that they dimerize with similar K-d values but the spectrum shifts toward the protonated state only with GFP. On excitation at 380 nm, the protonated GFPH emits at 510 nm via excited-state proton transfer, giving the appearance of extensive FRET. These findings have important implications for FRET measurements using GFP-type probes because they give rise to changes in donor and acceptor emission ratios through processes other than FRET and complicate the extraction of the true degree of energy transfer from experimental data. Furthermore, the unknown orientation factor prevents the distance of the lever arm swing from being derived from these FRET changes.

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19105086 BIOSIS NO.: 200600450481
Measurement of two caspase activities simultaneously in living cells by a novel dual FRET fluorescent indicator probe
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JOURNAL: Cytometry 69A (6): p477-486 JUN 2006 2006
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LANGUAGE: English

ABSTRACT: Background: A number of fluorescent caspase substrates and FRET-based indicators have been developed to study the in vivo activation of caspases, a conserved family of proteases critical in inflammatory, and apoptosis signaling pathways. To date, all substrates have measured only one caspase activity. Here, we describe a FRET-based probe for simultaneously measuring two distinct caspase activities in living cells.Methods: This probe consists of a CFP-YFP-mRFP %%%fusion%%% protein containing a caspase-3-cleavage motif, DEVD, between CFP and YFP and a caspase-6-cleavage site, VEID, between YFP and mRFP. DEVDase and VEIDase activities could be assessed simultaneously by monitoring din-finished FRET mediated by cleavage of either or both of these protease cleavage sites using flow cytometry.Results: DEVDase and VEIDase activities were completely inhibited by the pan-caspase inhibitor z-VAD-fmk and enhanced by DNA-damaging drugs or by anti-Fas stimulation. DEVD and VEID cleavage specificities were validated by using caspase-3-deficient MCF7-Fas cells and a caspase-6-specific inhibitor. Kinetic analysis with the FRET probe revealed that caspase-3 activation consistently, preceded caspase-6 by similar to 30 mill following induction of apoptosis.Conclusions: We have developed a novel FRET-based probe for simultaneous detection of two caspase activities in living cells Using flow cytometry. Simultaneous detection of two caspase activities using this probe has clearly provided information of the ordering of caspase-3 and -6 in the apoptotic pathway.
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19067325 BIOSIS NO.: 200600412720
Construction and evaluation of novel %%%fusion%%% proteins for targeted delivery of micro particles to cellulose surfaces
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JOURNAL: Biotechnology and Bioengineering 94 (4): p625-632 JUL 5 2006 2006
ISSN: 0006-3592
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ABSTRACT: The use of IgG antibodies and fragments has been limited to specific sectors of the biotechnology industry due to the high cost of producing large batches of product necessary for alternative applications. A novel class of Camelid antibodies, known as V-HH offer a more economical opportunity to meet a wider application in industry. In this study, we report the evaluation of four llama V-HH-cellulose binding domain %%%fusion%%% proteins displaying varying formats Of V-HH and CBD domains. Proteins were characterized in a targeted particle delivery system as a method of delivering agents such as perfume to laundry in the wash cycle. %%%Fusion%%% proteins were shown to be stable at high pH and in the presence of a detergent base. They were also shown to bind effectively to both the designated antigen, the azo-dye reactive-%%%red%%% 6 (either conjugated to BSA or attached to coacervate microparticles), and cellulose. Binding strength differences were observed between the different %%%fusion%%% protein formats using surface plasmon %%%resonance%%%. The effect of key laundry ingredients was also studied. Combining the %%%fusion%%% proteins and particles into a delivery and deposition study generated clear microscopy evidence for bifunctionality. Confirmation of this was validated by GC-MS analysis of retained fragrance. This research, reporting the construction and characterization of a variety of %%%fusion%%% proteins, illustrates that the single multidomain %%%fusion%%% protein route offers a new technology for successful targeted delivery of encapsulated benefit agents. Furthermore, the potential to modify or select for proteins to recognize a wide range of surfaces is also possible. (c) 2006 Wiley Periodicals, Inc.

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19015509 BIOSIS NO.: 200600360904
Targeted magnetic %%%resonance%%% imaging of scavidin-receptor in human umbilical vein endothelial cells in vitro
AUTHOR: Mantyla Tuomas; Hakumaki Juhana M (Reprint); Huhtala Tuulia; Narvanen Ale; Yla-Herttuala Seppo
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JOURNAL: Magnetic Resonance in Medicine 55 (4): p800-804 APR 2006 2006
ISSN: 0740-3194
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LANGUAGE: English

ABSTRACT: Current therapeutic approaches to treat cancer are often hampered by the lack of specificity of the drugs used for therapy. Scavidin, a novel %%%fusion%%% protein expressed on cell membranes, could be utilized for targeting of therapeutic molecules. Scavidin exploits the high binding affinity between avidin and biotin and is capable of mediating endocytosis of a bound ligand. In the current study we evaluated the efficiency of biotinylated ultrasmall superparamagnetic iron oxide (USPIO) particles in Scavidin-expressing human umbilical vein endothelial cell (HUVEC) cultures in vitro as a novel receptor-targeted magnetic %%%resonance%%% imaging contrast agent. Biotinylated USPIO (bUS- PIO) were targeted to Scavidin adenovirus-transcluced HUVECs in vitro.

Scavidin expressing cells were capable of binding and mediating endocytosis of the bUSPIO in vitro, which led to a significant decrease in T-2 relaxation times, and a loss of signal intensity in comparison to controls. The findings were confirmed with Prussian %%%blue%%% staining for iron and detection of Scavidin by bound biotinylated horseradish peroxidase. Our data shows that biotinylated ligands target specifically to Scavidin-expressing HUVEC in vitro. The utilization of Scavidin gene transfer ex vivo thus constitutes a platform for potential ligand delivery via cell therapy and time-independent imaging of biologic processes.

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19011937 BIOSIS NO.: 200600357332
Use of multigeneration-family molecular dog leukocyte antigen typing to select a hematopoietic cell transplant donor for a dog with T-cell lymphoma
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JOURNAL: Journal of the American Veterinary Medical Association 228 (5): p 728-732 MAR 1 2006 2006
ISSN: 0003-1488
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LANGUAGE: English

ABSTRACT: Case Description-A 7-year-old Golden Retriever was examined because of anorexia, lethargy, vomiting, and gradual weight loss.Clinical Findings-Splenomegaly, pancytopenia, high serum calcium concentration, and high alkaline phosphatase activity were detected. Magnetic %%%resonance%%% imaging revealed an enlarged mesenteric lymph node and increased signals from the bone marrow of the ilium and vertebral bodies. Histologic examination and immunophenotyping of biopsy specimens confirmed a stage V (b) T-cell malignant lymphoma.Treatment and Outcome-Clinical remission was attained by use of 2 chemotherapy cycles, followed by an allogeneic hematopoietic cell transplant performed at 18 weeks after diagnosis. A donor was identified by molecular dog leukocyte antigen typing methods. The patient was conditioned with 2 fractions of 4 Gy total body irradiation delivered 3 hours apart at 7 cGy/min, followed by an IV infusion of recombinant %%%canine%%% granulocyte colony-stimulating factor mobilized leukapheresis product and postgrafting immunosuppression with cyclosporine. %%%Chimerism%%% analyses revealed full donor engraftment that has been maintained for at least 58 weeks after transplant. Remission has been confirmed by normal results of serum thymidine kinase assays and the absence of peripheral blood clonal T-cell receptor gene rearrangements.Clinical Relevance-Systemic chemotherapy induces remissions-, however, most dogs succumb to disease recurrence because of multidrug resistance. Outcome of allogeneic hematopoietic cell transplantation in dogs can be excellent because of improved donor-recipient selection by use of molecular dog leukocyte antigen typing, compared with early attempts, and better

prevention of graft versus host disease, better supportive care, and substitution of peripheral blood mononuclear cells for bone marrow.

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18989361 BIOSIS NO.: 200600334756
Hypertrophy of the posterior longitudinal ligament in the thoracic spine
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JOURNAL: Spinal Cord 44 (3): p200-202 MAR 2006 2006
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RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Study design: This is a case report of a patient with hypertrophy of the posterior longitudinal ligament (HPLL) in the thoracic spine. This patient was followed for 10 years after surgery.Objectives: The purpose of this study was to report the long-term outcome of HPLL in the thoracic spine.Setting: Department of orthopedic surgery, Hiroshima %%%Red%%% Cross and Atomic-bomb Survivors Hospital, Hiroshima, Japan.Methods: A 58-year-old-woman with thoracic HPLL was reported. Magnetic %%%resonance%%% image (MRI) and computed tomography (CT) showed the expanded spinal cord compression from Th4 to Th12 due to HPLL. Anterior decompression and %%%fusion%%% (Th10-12) was performed. Histological findings of the surgical specimens showed thickening of the posterior longitudinal ligament with proliferation of chondroid tissue. The clinical outcome and the radiological findings (CT and MRI) were evaluated 10 years after surgery.Results: The patient was asymptomatic postoperatively. However, the subsequent CT examination revealed ossification of the previously hypertrophied posterior longitudinal ligament.Conclusions: HPLL in the thoracic spine is a rare pathological condition causing myelopathy. The results of this study support the hypothesis that HPLL is one of the prodromal conditions of HPLL.

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18776151 BIOSIS NO.: 200600121546
Intracellular interaction between syntaxin and Munc 18-1 revealed by fluorescence %%%resonance%%% energy transfer
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JOURNAL: Molecular Membrane Biology 22 (5): p401-410 SEP-OCT 2005 2005
ISSN: 0968-7688

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RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Neurosecretion is catalyzed by assembly of a soluble N-ethylmaleimide-sensitive %%%fusion%%% protein attachment protein receptor (SNARE)-complex composed of SNAP-25, synaptobrevin and syntaxin. Munc 18-1 is known to bind to syntaxin in vitro. This interaction prevents assembly of the SNARE-complex, but might also affect intracellular targeting of the proteins. We have fused syntaxin and Munc 18 to the yellow-(YFP) or cyan-fluorescence-protein (CFP) and expressed the constructs in CHO- and MDCK-cells. We have studied their localization with confocal microscopy and a possible protein-protein interaction with fluorescence-%%%resonance%%% energy transfer (FRET). YFP-syntaxin localizes to intracellular membranes. CFP-Munc 18 is present in the cytoplasm as expected for a protein lacking membrane targeting domains. However, Munc 18 is redirected to internal membranes when syntaxin is coexpressed, but only limited transport of the proteins to the plasma membrane was observed. An interaction between Munc 18 and syntaxin could be demonstrated by FRET using two methods, sensitized acceptor fluorescence and acceptor photobleaching. A mutation in syntaxin (L165A, E166A), which is known to inhibit binding to Munc 18 in vitro, prevents colocalization of the proteins and also the FRET signal. Thus, a protein-protein interaction between Munc 18 and syntaxin occurs on intracellular membranes, which is required but not sufficient for quantitative transport of both proteins to the plasma membrane.

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18659942 BIOSIS NO.: 200600005337
Micropatterning proteins on polyhydroxyalkanoate substrates by using the substrate binding domain as a %%%fusion%%% partner
AUTHOR: Park Jong Pill; Lee Kyung-Bok; Lee Seok Jae; Park Tae Jung; Kim Min Gon; Chung Bong Hyun; Lee Zee-Won; Choi Insung S; Lee Sang Yup (Reprint)
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JOURNAL: Biotechnology and Bioengineering 92 (2): p160-165 OCT 20 2005 2005
ISSN: 0006-3592
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ABSTRACT: A novel strategy for micropatterning proteins on the surface of polyhydroxyalkanoate (PHA) biopolymer by microcontact printing (mu CP) is described. The substrate binding domain (SBD) of the Pseudomonas stutzeri PHA depolymerase was used as a %%%fusion%%% partner for specifically immobilizing proteins on PHA substrate. Enhanced green fluorescent protein (EGFP) and %%%red%%% fluorescent protein (RFP) fused to the SBD could be specifically immobilized on the micropatterns of poly(3-hydroxybutyrate) and poly(3-hydroxybutyrate-co-3-hydroxyhexanoate). Laser scanning confocal microscopic studies suggested that two %%%fusion%%% proteins were

micropatterned in their functionally active forms. Also, antibody binding assay by surface plasmon %%%resonance%%% suggested that protein-protein interaction studies could be carried out using this system. (c) 2005 Wiley Periodicals, Inc.

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18585834 BIOSIS NO.: 200510280334
Ligand-induced dimer-tetramer transition during the activation of the cell surface epidermal growth factor receptor-A multidimensional microscopy analysis
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JOURNAL: Journal of Biological Chemistry 280 (34): p30392-30399 AUG 26 2005 2005
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ABSTRACT: The epidermal growth factor receptor (EGFR) is a member of the erbB tyrosine kinase family of receptors. For many years it has been believed that receptor activation occurs via a monomer-dimer transition that is associated with a conformational change to activate the kinase. However, little is known about the quaternary state of the receptor at normal levels of expression (< 105 receptors/cell). We employed multidimensional microscopy techniques to gain insight into the state of association of the human EGFR, in the absence and presence of ligand, on the surface of intact BaF/3 cells (50,000 receptors/cell). Image correlation microscopy of an EGFR-enhanced green fluorescent protein %%%chimera%%% was used to establish an average degree of aggregation on the submicron scale of 2.2 receptors/cluster in the absence of ligand increasing to 3.7 receptors/cluster in the presence of ligand. Energy transfer measurements between mixtures of %%%fluorescein%%% isothiocyanate-EGF and Alexa 555-EGF were performed using fluorescence lifetime imaging microscopy as a function of the donor: acceptor labeling ratio to gain insight into the spatial disposition of EGFR ligand binding sites on the nanometer scale. In the context of a two-state Forster %%%resonance%%% energy transfer (FRET)/non-FRET model, the data are consistent with a minimum transfer efficiency of 75% in the FRET population. The microscopy data are related to biophysical data on the EGFR in the A431 cell line and the three-dimensional structure of the ligated EGFR extracellular domain. In the context of a monomerdimer-oligomer model, the biophysical data are consistent with a significant fraction of ligated EGFR tetramers comprising two dimers juxtaposed in a side-by-side (or slightly staggered) arrangement. Our data are consistent with a specific higher order association of the ligand-bound EGFR on the nanometer scale and indicate the existence of distinct signaling entities beyond the level of the EGFR dimer which

could play an important role in receptor transactivation.

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18547086 BIOSIS NO.: 200510241586
Use of fluorescence %%%resonance%%% energy transfer to monitor the %%%fusion%%% between human Stratum corneum lipid liposomes and other phospholipid vesicles
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RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Different liposome formulations were prepared and their ability to fuse with liposomes obtained from human stratum corneum lipids (hSCLLs) was tested. %%%Resonance%%% energy transfer (RET) assay between N-(7-nitro-2,1,3-benzodiazol-4-yl) (N-NBD), as energy donor and %%%rhodamine%%%, N-(lissamine %%%Rhodamine%%% B sulfonyl), as energy acceptor, was developed to monitor the %%%fusion%%% process. The role of the lipid composition on the %%%fusion%%% or lipid mixing properties was investigated in order to obtain a suitable formulation for transdermal delivery. Our results showed that liposomes composed of PC:DOPE:Chol or DPPE:Chol:SA and also hSCLLs had a good ability to fuse with double labelled hSCLLs, compared to other liposome formulations. The efficacy of the process was of 29% for PC:DOPE:Chol liposomes, 26% for DPPE:Chol:SA liposomes and 11% for hSCLLs. The %%%fusion%%% between labelled hSCLLs and unlabelled ones increased with the temperature. Mixtures between liposomes prepared from commercially available lipids and hSCLLs showed a lower %%%fusion%%% efficacy at temperatures above 35 degrees C. Our studies confirmed the dependence of liposome fusogenicity on both the lipid composition and the temperature.

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18500975 BIOSIS NO.: 200510195475
Adding value to %%%fusion%%% proteins through covalent labelling
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JOURNAL: Current Opinion in Biotechnology 16 (4): p453-458 AUG 2005 2005
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ABSTRACT: Recombinant proteins are often expressed with an additional polypeptide the role of which is to aid purification, detection or functional studies. Recently, the role of these tags has been extended to mediate the labelling of the protein of interest with chemically diverse compounds. These approaches are of importance for protein science as they allow proteins to be equipped with properties that cannot be genetically encoded and also enable the use of a single %%%fusion%%% protein for several different applications. Several new approaches have been developed for the covalent labelling of %%%fusion%%% proteins both in living cells and in vitro.

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18280177 BIOSIS NO.: 200500187242
Glycan array screening reveals a candidate ligand for Siglec-8
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LANGUAGE: English

ABSTRACT: Sialic acid-binding immunoglobulin-like lectin 8 (Siglec-8) is selectively expressed on human eosinophils, basophils, and mast cells, where it regulates their function and survival. Previous studies demonstrated sialic acid-dependent binding of Siglec-8 but failed to reveal significant substructure specificity or high affinity of that binding. To test a broader range of potential ligands, a Siglec-8-Ig %%%chimeric%%% protein was tested for binding to 172 different glycan structures immobilized as biotinylated glycosides on a 384-well streptavidin-coated plate. Of these, apprx40 structures were sialylated Among these, avid binding was detected to a single defined glycan, NeuAcalpha-2-3(6-O-sulfo)Galbeta1-4(fucalpha1-3)GlcNAc, also referred to in the literature as 6'-sulfo-sLex. Notably, neither unsulfated sLex (NeuAcalpha2-3Galbeta1-4(Fucalpha1-3)GlcNAc) nor an isomer with the sulfate on the 6-position of the GlcNAc residue (6-sulfo-sLex, NeuAcalpha2-3Galbeta1-4(Fucalpha1-3)(6-O-sulfo)GlcNAc) supported detectable binding. Subsequent secondary screening was performed using surface plasmon %%%resonance%%%. Biotin glycosides immobilized on streptavidin biosensor chips were exposed to Siglec-8-Ig in solution. Whereas surfaces derivatized with sLex and 6-sulfo-sLex failed to support detectable Siglec-8 binding, 6'-sulfosLex supported significant binding with a Kd of 2.3 mum. In a separate test of binding specificity, aminopropyl glycosides were covalently immobilized at different concentrations on activated (N-hydroxysuccinimidyl) glass surfaces (Schott-Nexterion Slide H). Subsequent exposure to Siglec-8-Ig precomplexed with %%%fluorescein%%% isothiocyanate anti-human Fc resulted

in fluorescent signals at immobilized concentrations of 6'-sulfo-sLex of < 5 pmol/spot. In contrast, sLex and 6-sulfo-sLex did not support any Siglec-8 binding at the highest concentration tested (300 pmol/spot). We conclude that Siglec-8 binds preferentially to the sLex structure bearing an additional sulfate ester on the galactose 6-hydroxyl.

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18270100 BIOSIS NO.: 200500176836
A FlAsH-based FRET approach to determine G protein - coupled receptor activation in living cells
AUTHOR: Hoffmann Carsten; Gaietta Guido; Buenemann Moritz; Adams Stephen R; Oberdorff-Maass Silke; Behr Bjoern; Vilardaga Jean-Pierre; Tsien Roger Y; Eisman Mark H; Lohse Martin J (Reprint)
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JOURNAL: Nature Methods 2 (3): p171-176 March 2005 2005
MEDIUM: print
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LANGUAGE: English

ABSTRACT: Fluorescence %%%resonance%%% energy transfer (FRET) from cyan to yellow fluorescent proteins (CFP/YFP) is a welt-established method to monitor protein-protein interactions or conformational changes of individual proteins. But protein functions can be perturbed by %%%fusion%%% of large tags such as CFP and YFP. Here we use G protein-coupied receptor (GPCR) activation in living cells as a model, system to compare YFP with the small, membrane-permeant %%%fluorescein%%% derivative with two arsen-(III) substituents (%%%fluorescein%%% arsenical hairpin binder; FlAsH) targeted to a short tetracysteine sequence. Insertion of CFP and YFP into human adenosine A2A receptors allowed us to use FRET to monitor receptor activation but eliminated coupling to adenylyl cyclase. The CFP/FlAsH-tetracysteine system gave fivefold greater agonist-induced FRET signals, similar kinetics (time constant of 66-88 ms) and perfectly normal downstream signaling. Similar results were obtained for the mouse alpha2A-adrenergic receptor. Thus, FRET from CFP to FlAsH reports GPCR activation in living cells without disturbing receptor function and shows that the small size of the tetracysteine-biarsenical tag can be decisively advantageous.

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18260785 BIOSIS NO.: 200500167521
Improved "optical highlighter'' probes derived from discosoma %%%red%%% fluorescent protein
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JOURNAL: Biophysical Journal 88 (2): p1444-1457 February 2005 2005
MEDIUM: print
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ABSTRACT: The tetrameric %%%red%%% fluorescent protein, DsRed, undergoes a rapid %%%red%%% to green color change evoked by short wavelength (lambda 760 nm) femtosecond irradiation - a phenomenon that underpins the application of DsRed as an "optical highlighter'' probe for tracking live cells, organelles, and %%%fusion%%% proteins. This color change results from selective bleaching of the "mature'' %%%red%%%-emitting species of DsRed and an enhancement of emission from the "immature'' green species, likely caused by dequenching of fluorescence %%%resonance%%% energy transfer occurring within the protein tetramer. Here, we have examined the role of residues known to influence the rate and completeness of chromophore maturation on the cellular and biophysical properties of DsRed mutants. Surprisingly, a single amino acid mutation (N42Q) with increased basal green emission yet rapid chromophore maturation displayed a multiphoton-evoked color change that was brighter, more consistent, more vivid, and easier to evoke than DsRed, despite the larger proportion of green chromophores. Rapidly maturing mutants with more complete chromophore maturation, exhibited little color change and increased resistance to multiphoton bleaching. We describe improved optical and cell biological properties for two DsRed-derived variants which we showcase in photolabeling studies, and discuss these data in terms of implications for fluorescence %%%resonance%%% energy transfer-based probes.

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18240035 BIOSIS NO.: 200500147100
Fluorescence properties of green fluorescent protein FRET pairs concatenated with the small G protein, Rac, and its interacting domain of the kinase, p21-activated kinase
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ISSN: 1540-658X _(ISSN print)
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LANGUAGE: English

ABSTRACT: Many diseases are caused by aberrant cell signalling controlled by intracellular protein - protein interactions. Inhibitors of such interactions thus have enormous potential as chemotherapeutic agents. It is advantageous to test for such inhibitors using cell- based screens in

which modulation of the interaction gives a rapid response. Fluorescence %%%resonance%%% energy transfer (FRET) systems, based on interacting donor and acceptor green fluorescent proteins (GFPs), have potential in such screens. Here, we describe experiments aimed at using a FRET system to monitor the interaction between the small G protein Rac and a region of its binding partner, the Ser/ Thr kinase, p21- activated kinase (PAK). Initial attempts to use a previously described construct, enhanced GFP- PAK- enhanced %%%blue%%% fluorescent protein, failed because of the difficulty of obtaining equal and high expression levels of both the %%%fusion%%% protein and Rac in mammalian cells. Here, three proteins in which Rac, PAK, and the two GFPs were concatenated in different combinations on a single protein were expressed and characterised. In each construct, however, intramolecular interaction of PAK and Rac was observed. As this was of extremely high affinity, presumably because of entropy effects from the interacting partners being tethered, these molecules were not suitable for detection of inhibitors of the interaction. Molecular modelling was used to investigate the way in which the concatenated constructs might form intramolecular interactions. As this explained key properties of these proteins, it is likely that this approach could be used to design constructs where the unwanted intramolecular protein - protein interactions are prevented, whilst allowing the desired intermolecular Rac/ PAK interaction. This would provide constructs that are useable for drug discovery.

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18239679 BIOSIS NO.: 200500146744
Three-chromophore FRET microscopy to analyze multiprotein interactions in living cells
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JOURNAL: Nature Methods 1 (3): p209-217 December 2004 2004
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LANGUAGE: English

ABSTRACT: Nearly every major process in a cell is carried out by assemblies of multiple dynamically interacting protein molecules. To study multi-protein interactions within such molecular machineries, we have developed a fluorescence microscopy method called three-chromophore fluorescence %%%resonance%%% energy transfer (3-FRET). This method allows analysis of three mutually dependent energy transfer processes between the fluorescent labels, such as cyan, yellow and monomeric %%%red%%% fluorescent proteins. Here, we describe both theoretical and experimental approaches that discriminate the parallel versus the sequential energy transfer processes in the 3-FRET system. These approaches were established in vitro and in cultured mammalian cells, using %%%chimeric%%% proteins consisting of two or three fluorescent proteins linked together. The 3-FRET microscopy was further applied to the analysis of three-protein interactions in the constitutive and

activation-dependent complexes in single endosomal compartments. These data highlight the potential of 3-FRET microscopy in studies of spatial and temporal regulation of signaling processes in living cells.

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18178876 BIOSIS NO.: 200500085941
EosFP, a fluorescent marker protein with UV-inducible green-to-%%%red%%% fluorescence conversion
AUTHOR: Wiedenmann Joerg; Ivanchenko Sergey; Oswald Franz; Schmitt Florian; Roecker Carlheinz; Salih Anya; Spindler Klaus-Dieter; Nienhaus G Ulrich (Reprint)
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JOURNAL: Proceedings of the National Academy of Sciences of the United States of America 101 (45): p15905-15910 November 9, 2004 2004
MEDIUM: print
ISSN: 0027-8424 _(ISSN print)
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: A gene encoding a fluorescent protein from the stony coral Lobophyllia hemprichii has been cloned in Escherichia coli and characterized by biochemical and biophysical methods. The protein, which we named EosFP, emits strong green fluorescence (516 nm) that changes to %%%red%%% (581 nm) upon near-UV irradiation at apprxeq390 nm because of a photo-induced modification involving a break in the peptide backbone next to the chromophore. Single-molecule fluorescence spectroscopy shows that the wild type of EosFP is tetrameric, with strong Forster %%%resonance%%% coupling among the individual fluorophores. We succeeded in breaking up the tetramer into AB and AC subunit dimers by introducing the single point mutations V123T and T158H, respectively, and the combination of both mutations yielded functional monomers. %%%Fusion%%% constructs with a variety of proteins were prepared and expressed in human cells, showing that normal biological functions were retained. The possibility to locally change the emission wavelength by focused UV light makes EosFP a superb marker for experiments aimed at tracking the movements of biomolecules within the living cell.

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18149452 BIOSIS NO.: 200500056517
A trimeric HIV-1 %%%fusion%%% peptide construct which does not self-associate in aqueous solution end which has 15-fold higher membrane %%%fusion%%% rate
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18091020 BIOSIS NO.: 200400472249
Specific antitumor targetable beta-cyclodextrin-poly(ethylene glycol)-folic acid drug delivery bioconjugate
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JOURNAL: Bioconjugate Chemistry 15 (5): p997-1004 September 2004 2004
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ABSTRACT: The tumor targeting properties of a new drug carrier synthesized by bioconjugation of folic acid (FA) to beta-cyclodextrins through a poly(ethylene glycol) (PEG) spacer (CD-PEG-FA) were investigated. Surface plasmon %%%resonance%%% demonstrated that CD-PEG-FA specifically interacts with immobilized folate binding protein (FBP) while the naked beta-cyclodextrins do not display any specific interaction. In vitro studies demonstrated that CD-PEG-FA was devoid of cell toxicity. (3H)-folic acid/CD-PEG-FA competition binding investigations performed with folate receptor overexpressing human epidermal carcinoma KB cells showed that CD-PEG-FA had about 14 times lower tumor cell binding capacity than free folic acid. The carrier cell trafficking properties were investigated using %%%rhodamine%%%-B as fluorescent probe, which possesses 3000 and 4580 M-1 inclusion constants for CD-PEG-FA and beta-cyclodextrins, respectively. Cell-associated fluorescence measurements showed that CD-PEG-FA does not promote the %%%rhodamine%%%-B uptake into non-folate receptor expressing human lung carcinoma MCF7 cells while 19% higher accumulation in KB cells was found with respect to %%%rhodamine%%%-B loaded beta-cyclodextrins. Confocal laser scanning microscopy indicated the presence of cytosolic %%%red%%% fluorescent spots after 2 h of incubation of KB cells with %%%rhodamine%%%-B included CD-PEG-FA. The fluorescent dye resided primarily in small spots, namely, endosomes and multivesicular bodies. At 1 h after pulsed incubation, wider %%%red%%% fluorescent cellular structures appeared as a %%%fusion%%% of previous structures.

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18091018 BIOSIS NO.: 200400472247
Detection of apoptosis using the C2A domain of synaptotagmin I
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ABSTRACT: Binding of annexin V or the C2A domain of synaptotagmin I to phosphatidylserine expressed on the surface of apoptotic cells can, when labeled with appropriate probe molecules, be used to detect the presence of apoptosis using radionuclide, magnetic %%%resonance%%%, and optical imaging techniques. The preparation of a biotinylated C2A-GST %%%fusion%%% protein is described, and its capability, when used in conjunction with %%%fluorescein%%%-labeled streptavidin, of detecting apoptotic cells by flow cytometry is compared directly with the performance of a commercial preparation of %%%fluorescein%%%-labeled annexin V. Biotinylated C2A-GST, when used in conjunction with streptavidin-conjugated superparamagnetic iron oxide nanoparticles or Gd-chelate-avidin conjugates, was shown to be capable of detecting apoptotic cells using T2-weighted or T1-weighted magnetic %%%resonance%%% imaging experiments, respectively.

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18021827 BIOSIS NO.: 200400392616
Consequences of nonlytic membrane perturbation to the translocation of the cell penetrating peptide pep-1 in lipidic vesicles
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ABSTRACT: The action of the cell penetrating pep-1 at the molecular level is not clearly understood. The ability of the peptide to induce (1) vesicle aggregation, (2) lipidic %%%fusion%%%, (3) anionic lipid segregation, (4) pore or other lytic structure formation, (5) asymmetric lipidic flip-flop, and (6) peptide translocation across the bilayers in large unilamellar vesicles was studied using photophysical methodologies mainly related to fluorescence spectroscopy. Neflometry and turbidimetry

techniques show that clustering of vesicles occurs in the presence of the peptide in a concentration- and anionic lipid content-dependent manner. Results from Forster %%%resonance%%% energy transfer-based methodologies prove lipidic %%%fusion%%% and anionic lipid segregation, but no evidence for pores or other lytic structures was found. Asymmetric lipid flip-flop was not detected either. A specific method related to the quenching of the %%%rhodamine%%%-labeled lipids by pep-1 was developed to study the eventual translocation of the peptide. Translocation does not occur in symmetrical neutral and negatively charged vesicles, except when a valinomycin-induced transmembrane potential exists. Our work strongly suggests that the main driving force for peptide translocation is charge asymmetry between the outer and inner leaflet of biological membranes and reveals that pep-1 is able to perturb membranes without being cytotoxic. This nonlytic perturbation is probably mandatory for translocation to occur.

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18008043 BIOSIS NO.: 200400378832
%%%Fusion%%% of Aequorea victoria GFP and aequorin provides their Ca2+-induced interaction that results in %%%red%%% shift of GFP absorption and efficient bioluminescence energy transfer
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JOURNAL: Biochemical and Biophysical Research Communications 320 (3): p 703-711 July 30, 2004 2004
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ABSTRACT: The bioluminescence emitted by Aequorea victoria jellyfish is greenish while its single bioluminescent photoprotein aequorin emits %%%blue%%% light. This phenomenon may be explained by a bioluminescence %%%resonance%%% energy transfer (BRET) from aequorin chromophore to green fluorescent protein (GFP) co-localized with it. However, a slight overlapping of the aequorin bioluminescence spectrum with the GFP absorption spectrum and the absence of marked interaction between these proteins in vitro pose a question on the mechanism providing the efficient BRET in A. uictoria. Here we report the in vitro study of BRET between homologous Ca2+-activated photoproteins, aequorin or obelin (Obelia longissima), as bioluminescence energy donors, and GFP, as an acceptor. The fusions containing donor and acceptor proteins linked by a 19 aa peptide were purified after expressing their genes in Escherichia coli cells. It was shown that the GFP-aequorin %%%fusion%%% has a significantly greater BRET efficiency, compared to the GFP-obelin %%%fusion%%%. Two main factors responsible for the difference in BRET efficiency of these fusions were revealed. First, it is the presence of Ca2+-induced interaction between the donor and acceptor in the

aequorin-containing %%%fusion%%% and the absence of the interaction in the obelin-containing %%%fusion%%%. Second, it is a %%%red%%% shift of GFP absorption toward better overlapping with aequorin bioluminescence induced by the interaction of aequorin with GFP. Since the connection of the two proteins in vitro mimics their proximity in vivo, Ca2+-induced interaction between aequorin and GFP may occur in A. victoria jellyfish providing efficient BRET in this organism. Copyright 2004 Elsevier Inc. All rights reserved.

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17947487 BIOSIS NO.: 200400318244
Monitoring caspase activity in living cells using fluorescent proteins and flow cytometry
AUTHOR: He Liusheng (Reprint); Wu Xiaoli; Meylan Francoise; Olson Douglas P ; Simone James; Hewgill Derek; Siegel Richard; Lipsky Peter E
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ABSTRACT: A molecular probe was developed to monitor caspase activity in living cells by flow cytometry. It consists of CFP and YFP with a peptide linker containing two caspase-cleavage sites (LEVD). Its expression resulted in intense fluorescence %%%resonance%%% energy transfer (FRET), whereas cleavage of this linker by caspases eliminated FRET because of physical separation of the CFP and YFP moieties. Using flow cytometry, cells expressing this probe exhibited two patterns, strong FRET and diminished or absent FRET. The appearance of diminished FRET was inhibited by a pan-caspase inhibitor z-VAD or D->A mutations in the LEVD sequence and was markedly increased by apoptosis-inducing agents, etoposide and camptothecin, or overexpression of a caspase 8-%%%red%%% fluorescent protein %%%fusion%%% protein. Importantly, this probe's ability to monitor caspase activity was comparable with results obtained with fluorogenic substrates or fluorochrome-labeled inhibitors of caspases. Specific caspase inhibitors indicated the probe was highly sensitive to cleavage by caspase 6 and 8, less sensitive to caspase 4, and resistant to other caspases. Activation of caspase 8 by Fas engagement markedly increased the probe's cleavage, whereas treatment of caspase 8-deficient cells with anti-Fas did not increase cleavage. However, staurosporine induced cleavage of the probe in caspase 8-deficient cells by a mechanism that was inhibited by overexpression of bcl-x. Taken together, the data indicate that this caspase-sensitive probe can be used to monitor the basal and apoptosis-related activities of caspases. including an initiator caspase, caspase 8, and effector caspases, such as caspase 6.

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17924841 BIOSIS NO.: 200400295598
%%%Red%%%-edge anisotropy microscopy enables dynamic imaging of homo-FRET between green fluorescent proteins in cells
AUTHOR: Squire Anthony; Verveer Peter J; Rocks Oliver; Bastiaens Philippe I H (Reprint)
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ABSTRACT: Steady-state fluorescence anisotropy measurements can be used to detect fluorescence %%%resonance%%% energy transfer (FRET) between identical fluorophores (homo-FRET). However, the contribution of homo-FRET to the steady-state anisotropy must be discerned from those due to the orientational distribution and rotational diffusion, which so far has required photobleaching controls, largely precluding dynamic measurements in live cells. We describe a variation of steady-state anisotropy microscopy in which the contribution of homo-FRET is dynamically isolated from the total anisotropy by exploiting the loss of energy transfer that occurs at %%%red%%%-edge excitation. Excitation of enhanced green fluorescent protein (EGFP) at the %%%red%%%-edge of its absorption band shows the shift in the emission spectrum compared to main-band excitation that is characteristic for photo-selection of static low energy So-S, transitions that fail to exhibit FRET. An experimental setup for steady-state fluorescent anisotropy microscopy is described that can be used to acquire anisotropy images in live cells at main-band and %%%red%%%-edge excitation of EGFP. We demonstrate in live cells homo-FRET suppression of protein %%%fusion%%% constructs that consist of two and three EGFP molecules connected by short linkers. This methodology represents a novel approach for the dynamic measurement of homo-FRET in live cells that will be of utility in the biological sciences to detect oligomerization and concentration dependent interactions between identically labeled molecules. Copyright 2003 Elsevier Inc. All rights reserved.

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17770237 BIOSIS NO.: 200400137591
Improved transposon-based tools for generating random GFP %%%fusion%%% proteins.
AUTHOR: Sheridan Douglas L (Reprint); Hughes Thomas E
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JOURNAL: Biophysical Journal 86 (1): p626a-627a January 2004 2004
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RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Transposons can be used to create functional fluorescent %%%fusion%%% proteins by randomly inserting DNA encoding the green fluorescent protein (GFP) within another coding sequence (Sheridan et al., BMC Neurosci., 3:7). However, the creation of multi-colored libraries for co-expression or fluorescence %%%resonance%%% energy transfer (FRET) still requires either multiple transpositions or tedious subcloning. To address this limitation, we developed two new transposons encoding multiple fluorescent proteins. The first design, the Either-Or transposon (ltbbracEYORrtbbrac2), carries the yellow and cyan fluorescent proteins (YFP and CFP) in a parallel orientation. The fluorescent protein coding sequences are each flanked by two rare 8 bp restriction sites in identical overlapping cassettes, such that alternate digestion and re-ligation of in-frame insertions produces identical full-length YFP or CFP %%%fusion%%% proteins. To test this design, we targeted the integral membrane protein Prestin, producing both CFP and YFP fusions at 13 unique sites. The second transposon, the Double Barrel transposon (ltbbracDBTrtbbrac), encodes green and %%%red%%% fluorescent proteins (GFP and DsRed, respectively) positioned anti-parallel to one another. This design could potentially double the efficiency of %%%fusion%%% protein generation, from 1:6 to 1:3, since insertions within another coding sequence are independent of orientation. In addition, the two fluorophores use different relative open reading frames through the transposon ends, thereby increasing the total number of potential in-frame insertion sites in a given coding sequence to 2:3. To test ltbbracDBTrtbbrac, we transposed the cDNA encoding the type 1 IP3 receptor, generating 41 full-length GFP fusions and 2 DsRed fusions.

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17756213 BIOSIS NO.: 200400126970
Fluorescence energy transfer within the Ca-ATPase using genetically encoded fluorescent probes.
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AUTHOR ADDRESS: Biochemistry, Molecular Biology, and Biophysics, University of Minnesota, Minneapolis, MN, USA**USA
JOURNAL: Biophysical Journal 86 (1): p243a January 2004 2004
MEDIUM: print
CONFERENCE/MEETING: 48th Annual Meeting of the Biophysical Society Baltimore, MD, USA February 14-18, 2004; 20040214
SPONSOR: Biophysical Society
ISSN: 0006-3495 _(ISSN print)
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: We have used genetically encoded fluorophores to create specific labeling sites on the Ca-ATPase from sarcoplasmic reticulum (SERCA) in

order to investigate functional domain movements during the enzymatic cycle. These experiments are designed to test the hypothesis, based on x-ray crystallography, that the nucleotide binding (N) and actuator (A) domains of SERCA undergo a dramatic rearrangement, moving several nm closer to each other, upon Ca binding to SERCA. Previously, we created a cDNA construct allowing for the %%%fusion%%% of cyan fluorescent protein (CFP) to the N-terminus of SERCA in the A domain. Current advances in the enhancement of fluorescent proteins have revealed an A206K mutation that favors the monomeric form of CFP. A206K-CFP-SERCA was over-expressed in Sf21 insect cells using the baculovirus system. To detect domain movements using fluorescence %%%resonance%%% energy transfer (FRET), A206K-CFP-SERCA was selectively labeled with %%%fluorescein%%% isothiocyanate (FITC) at Lys515 in the N domain. FRET (27% efficiency) was observed between A206K-CFP (donor) and FITC (acceptor), indicating a distance of 65 ANG between probes on the A and N domains, but no significant change was observed upon Ca binding. We propose that ATP binding (inhibited by FITC at Lys515) is required for large-scale domain movements in SERCA. To test this hypothesis, we have engineered several tetra-cysteine labeling sites (CCXXCC) outside the ATP binding pocket on the N domain. These sites provide for site-specific labeling with the novel fluorescent probe FlAsH (%%%fluorescein%%% bis-arsenical helix/hairpin binder). This will allow FRET to be used to detect changes in the interdomain distance in response to ATP and other nucleotides.

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17755766 BIOSIS NO.: 200400126523
Conformational changes associated with Ca2+ binding to the Ca2+-regulatory domain of NCX1.
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AUTHOR ADDRESS: Departments of Physiology, Chemistry and Biochemistry, and Medicine, UCLA, Los Angeles, CA, USA**USA
JOURNAL: Biophysical Journal 86 (1): p101a January 2004 2004
MEDIUM: print
CONFERENCE/MEETING: 48th Annual Meeting of the Biophysical Society Baltimore, MD, USA February 14-18, 2004; 20040214
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RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The Na/Ca exchanger, NCX1, is regulated at a Ca2+ regulatory site located in the large cytoplasmic loop. We have expressed this domain in E. coli cells as a His6 tagged %%%fusion%%% protein and purified it to apparent homogeneity on a Ni-NTA affinity column. The %%%fusion%%% protein displays Ca2+-induced shifts in mobility upon SDS-PAGE and binding of 45Ca2+ on nitrocellulose overlays. By equilibrium dialysis, 2 mols of Ca2+ bind per mol of protein with an apparent affinity of about 0.1 muM. We have been using this %%%fusion%%% protein to study conformational changes associated with Ca2+-binding. The Ca2+-bound form of the domain displays a more open conformation than the unbound form. On ultracentrifugation, the Ca2+-bound form has an observed Svedberg

constant of 1.9-1.95 and in the presence of EGTA, it increases to 2.1. Also, the Ca2+ unbound, but not the Ca2+ bound, form passes through a MW cutoff filter of 30,000 kDa upon ultrafiltration. The domain contains 3 cysteine residues that can be modified with the fluorescent probe AEDANS. The rate and extent of labelling in the presence of EGTA is much greater than in the presence of Ca2+. We have introduced tryptophan residues at several locations in the domain and examined the effect of Ca2+ on tryptophan fluorescence. Two mutants display Ca2+-induced quenching and one displays a Ca2+-induced enhancement of fluorescence. Two mutants show Ca2+-induced %%%blue%%% shifts. We are using FRET to estimate the distance between introduced tryptophans and AEDANS-tagged cysteines.

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17744783 BIOSIS NO.: 200400114489
Structural dynamics of the sarcoplasmic reticulum calcium pump using bis-arsenical %%%fluorescein%%% (FLASH) at engineered tetra-cysteine motifs.
AUTHOR: Autry Joseph M (Reprint); Winters Deborah L (Reprint); Thomas David D (Reprint)
AUTHOR ADDRESS: Biochemistry, Molecular Biology, and Biophysics, University of Minnesota, Minneapolis, MN, USA**USA
JOURNAL: Biophysical Journal 86 (1): p192a January 2004 2004
MEDIUM: print
CONFERENCE/MEETING: 48th Annual Meeting of the Biophysical Society Baltimore, MD, USA February 14-18, 2004; 20040214
SPONSOR: Biophysical Society
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DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: FLASH (%%%fluorescein%%% bis-arsenical helix/hairpin binder) is a novel fluorescent probe that specifically reacts with tetra-cysteine motifs (CCXXCC) in proteins. We utilized site-directed FLASH labeling to investigate the structural dynamics involved in active calcium transport by the sarcoplasmic reticulum calcium pump (SERCA). Five tetra-cysteine motifs were individually created in the cytoplasmic headpiece of SERCA, with four sites in the nucleotide-binding (N) domain (starting at residues 397, 535, 574, or 581) and one site in the phosphorylation (P) domain (starting at residue 670). SERCA tetra-cysteine mutants were expressed using the baculovirus system and labeled in Sf21 cell microsomes with FLASH (provided by Nariman Naber and Roger Cooke). Ingel fluorescence imaging demonstrated that all five engineered tetra-cysteine sites reacted with FLASH, but that wild-type SERCA did not. SERCA mutants labeled with FLASH showed strong fluorescence that was insensitive to calcium, nucleotide, phosphate, or thapsigargin. To detect domain movements using fluorescence %%%resonance%%% energy transfer (FRET), two approaches were taken to introduce donor/acceptor probe pairs into the cytoplasmic headpiece of SERCA: 1) tetra-cysteine mutants were labeled with FLASH in the N domain and IAEDANS in the P domain, and 2) a tetra-cysteine motif was created and labeled in the P domain of CFP-SERCA, a %%%fusion%%% protein with cyan fluorescent protein (CFP) attached to the N-terminus of SERCA in the actuator (A) domain. FRET

experiments with doubly-labeled SERCA (IAEDANS/FLASH and CFP/FLASH) are in progress to determine the distance between domains (P to N and A to P, respectively) as a function of calcium and nucleotide.

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17673782 BIOSIS NO.: 200400044539
Monounsaturated fatty acid oligomerization is responsible for the agglutination activity of heated virgin olive oil.
AUTHOR: Patrikios Ioannis S (Reprint); Patsalis Philippos C
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JOURNAL: Food Research International 36 (9-10): p985-990 2003 2003
MEDIUM: print
ISSN: 0963-9969
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The present study focuses on the isolation, purification and characterization of a molecule formed when virgin olive oil is heated at 130 degreeC for 24 h in air, that is found to be a strong agglutinin. The hemagglutinating activity of the novel molecule isolated from the heated olive oil was evaluated against human %%%red%%% blood cells (RBCs). Thin layer chromatography (TLC) of the oil mixture showed appearance of high molecular weight molecules, dimers and polymers. 13C nuclear magnetic %%%resonance%%% (NMR) and mass chromatography-mass spectroscopy (GC-MS) were used for structure elucidation. A linear oligomerization of monounsaturated fatty acids is involved. Light microscopy and electron microscopy were used to characterize and visualize the agglutination process. Agglutination without lysis or %%%fusion%%% was observed. The unheated olive oil and the isolated compound were also tested in-vitro against normal and malignant colon and breast cells. The results showed the highest reduction of tumor cells with the isolated novel compound. We conclude that virgin olive oil when heated in air produces oligomerization/polymerization of free unsaturated fatty acid possibly oleic acid (OA) that is a strong hemagglutinin against human RBCs with possible anti-cancer properties but with unknown nutritional effects on human health.

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17659117 BIOSIS NO.: 200400029874
Fluorescence evidence for a loose self-assembly of the %%%fusion%%% peptide of influenza virus HA2 in the lipid bilayer.
AUTHOR: Cheng Shu-Fang; Kantchev Assen B; Chang Ding-Kwo (Reprint)
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JOURNAL: Molecular Membrane Biology 20 (4): p345-351 October-December 2003

2003
MEDIUM: print
ISSN: 0968-7688
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Steady state fluorescence experiments were performed on a 25-mar synthetic peptide incorporated in the phospholipid vesicle to study the role of oligomerization of the %%%fusion%%% peptide in membrane %%%fusion%%%. It was found from fluorescence %%%resonance%%% energy transfer (FRET) that the extent of lipid mixing and the initial mixing rate varied with the %%%fusion%%% peptide concentration in a higher than linear fashion, indicating that the peptide promoted membrane mixing as oligomers. Results of self-quenching of the %%%Rhodamine%%% (Rho) in Rho-labelled peptide incorporated in the phospholipid bilayer indicated that the peptide molecules assembled in the bilayer with an order higher than dimer. The data also revealed that the peptides were not tightly packed in the membrane. Binding affinity measurement monitored by the NBD fluorescence intensity on the fluorophore-labelled %%%fusion%%% peptide supports the notion of self-association of the peptide in the vesicular dispersion. In the sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) experiments, a diffuse band with apparent molecular mass close to a dimeric species of the wild type %%%fusion%%% peptide suggested that the %%%fusion%%% peptides formed loose oligomers under the influence of SDS detergent in the electric field. The result is in contrast to a less %%%fusion%%%-active variant which appears to exhibit less propensity for self-association.

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17480234 BIOSIS NO.: 200300437268
Lipid bilayer vesicle %%%fusion%%%: Intermediates captured by high-speed microfluorescence spectroscopy.
AUTHOR: Lei Guohua; MacDonald Robert C (Reprint)
AUTHOR ADDRESS: Dept. of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston, IL, 60208, USA**USA
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JOURNAL: Biophysical Journal 85 (3): p1585-1599 September 2003 2003
MEDIUM: print
ISSN: 0006-3495 _(ISSN print)
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The %%%fusion%%% of lipid bilayers can be visualized under the fluorescence microscope, but the process is very fast and requires special techniques for its study. It is reported here that vesicle %%%fusion%%% is susceptible to analysis by microspectrofluorometry and that for the first time, the entire %%%fusion%%% process has been captured. In the case of giant (>10-mum diameter) bilayer vesicles having a high density of opposite charge, %%%fusion%%% proceeds through stages of adhesion, flattening, hemifusion, elimination of the intervening septum, and uptake of excess membrane to generate a spherical product

very rapidly. These investigations became possible with a fluorescence microscope that was modified for recording of images simultaneously with the collection of fluorescence emission spectra from many (>100) positions along the %%%fusion%%% axis. Positively-charged vesicles, composed of O-ethylphosphatidylcholine and dioleoylphosphatidylcholine, were labeled with a carbocyanine fluorophore. Negatively-charged vesicles, composed of dioleoylphosphatidylglycerol and dioleoylphosphatidylcholine, were labeled with a %%%rhodamine%%% fluorophore that is a %%%resonance%%% energy transfer acceptor from the carbocyanine fluorophore. An electrophoretic chamber allowed selection of pairs of vesicles to be brought into contact and examined. Spectral changes along the axis of %%%fusion%%% were captured at high speed (a few ms/frame) by operating a sensitive digital camera in the virtual-chip mode, a software/hardware procedure that permits rapid readout of selected regions of interest and by pixel binning along the spectral direction. Simultaneously, color images were collected at video rates (30 frame/s). Comparison of the spectra and images revealed that vesicle %%%fusion%%% typically passes through a hemifusion stage and that the time from vesicle contact to %%%fusion%%% is <10 ms. Fluorescence spectra are well suited to rapid collection in the virtual-chip mode because spectra (in contrast to images) are accurately characterized with a relatively small number of points and interfering signals can be removed by judicious choice of barrier filters. The system should be especially well-suited to phenomena exhibiting rapid fluorescence change along an axis; under optimal conditions, it is possible to obtain sets of spectra (wavelength range of apprx150 nm) at >100 positions along a line at rates >1000 frames/s with a spectral resolution of apprx10 nm and spatial resolution at the limit of the light microscope (apprx0.2 mum).

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17468271 BIOSIS NO.: 200300423115
Fluorescence energy transfer within the Ca-ATPase using cyan fluorescent protein fused to the N-terminus.
AUTHOR: Winters Deborah L (Reprint); Autry Joseph M (Reprint); Thomas David D (Reprint)
AUTHOR ADDRESS: Department of Biochemistry, Molecular Biology, and Biophysics, University of Minnesota, Minneapolis, MN, 55455, USA**USA
JOURNAL: Biophysical Journal 84 (2 Part 2): p264a February 2003 2003
MEDIUM: print
CONFERENCE/MEETING: 47th Annual Meeting of the Biophysical Society San Antonio, TX, USA March 01-05, 2003; 20030301
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17395146 BIOSIS NO.: 200300353865

Efficiently folding and circularly permuted variants of the Sapphire mutant of GFP.
AUTHOR: Zapata-Hommer Otilia; Griesbeck Oliver (Reprint)
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JOURNAL: BMC Biotechnology 3 (5 Cited June 27, 2003): May 22, 2003 2003
MEDIUM: online
ISSN: 1472-6750 _(ISSN online)
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Background: The green fluorescent protein (GFP) has been widely used in cell biology as a marker of gene expression, label of cellular structures, %%%fusion%%% tag or as a crucial constituent of genetically encoded biosensors. Mutagenesis of the wildtype gene has yielded a number of improved variants such as EGFP or colour variants suitable for fluorescence %%%resonance%%% energy transfer (FRET). However, folding of some of these mutants is still a problem when targeted to certain organelles or fused to other proteins. Results: By directed rational mutagenesis, we have produced a new variant of the Sapphire mutant of GFP with improved folding properties that turns out to be especially beneficial when expressed within organelles or as a %%%fusion%%% tag. Its absorption spectrum is pH-stable and the pKa of its emission is 4.9, making it very resistant to pH perturbation inside cells. Conclusion: "T-Sapphire" and its circular permutations can be used as labels of proteins or cellular structures and as FRET donors in combination with %%%red%%%-fluorescent acceptor proteins such as DsRed, making it possible to completely separate donor and acceptor excitation and emission in intensity-based FRET experiments.

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17352667 BIOSIS NO.: 200300310156
The BRET2/arrestin assay in stable recombinant cells: A platform to screen for compounds that interact with G Protein-Coupled Receptors (GPCRS).
AUTHOR: Bertrand Lucie (Reprint); Parent Stephane (Reprint); Caron Mireille (Reprint); Legault Mireille (Reprint); Joly Erik (Reprint); Angers Stephane; Bouvier Michel; Brown Mike (Reprint); Houle Benoit (Reprint); Menard Luc (Reprint)
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JOURNAL: Journal of Receptors and Signal Transduction 22 (1-4): p533-541 February-May-August-November 2002 2002
MEDIUM: print
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: In BRET2 (Bioluminescence %%%Resonance%%% Energy Transfer), a Renilla luciferase (RLuc) is used as the donor protein, while a Green Fluorescent Protein (GFP2) is used as the acceptor protein. In the presence of the cell permeable substrate DeepBlueCTM, RLuc emits

%%%blue%%% light at 395 nm. If the GFP2 is brought into close proximity to RLuc via a specific biomolecular interaction, the GFP2 will absorb the %%%blue%%% light energy and reemit green light at 510 nm. BRET2 signals are therefore easily determined by measuring the ratio of green over %%%blue%%% light (510/395 nm) using appropriate dual channel luminometry instruments (e.g., FusionTM Universal Microplate Analyzer, Packard BioScience). Since no light source is required for BRET2 assays, the technology does not suffer from high fluorescent background or photobleaching, the common problems associated with standard FRET-based assays. Using BRET2, we developed a generic G Protein-Coupled Receptor (GPCR) assay based on the observation that activation of the majority of GPCRs by agonists leads to the interaction of beta-arrestin (a protein that is involved in receptor desensitization and sequestration) with the receptor. We established a cell line stably expressing the GFP2: beta-arrestin 2 %%%fusion%%% protein, and showed that it can be used to monitor the activation of various transiently expressed GPCRs, in BRET2/arrestin assays. In addition, using the HEK 293/GFP2: beta-arrestin 2 cell line as a recipient, we generated a double-stable line co-expressing the vasopressin 2 receptor (V2R) fused to RLuc (V2R: RLuc) and used it for the pharmacological characterization of compounds in BRET2/arrestin assays. This approach yields genuine pharmacology and supports the BRET2/arrestin assay as a tool that can be used with recombinant cell lines to characterize ligand-GPCR interactions which can be applied to ligand identification for orphan receptors.

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17277080 BIOSIS NO.: 200300233880
%%%Blue%%% light perception in plants: Detection and characterization of a light-induced neutral flavin radical in a C450A mutant of phototropin.
AUTHOR: Kay Christopher W M; Schleicher Erik; Kuppig Andreas; Hofner Heidi; Ruediger Wolfhart; Schleicher Michael; Fischer Markus; Bacher Adelbert; Weber Stefan (Reprint); Richter Gerald
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JOURNAL: Journal of Biological Chemistry 278 (13): p10973-10982 March 28, 2003 2003
MEDIUM: print
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The LOV2 domain of Avena sativa phototropin and its C450A mutant were expressed as recombinant %%%fusion%%% proteins and were examined by optical spectroscopy, electron paramagnetic %%%resonance%%%, and electron-nuclear double %%%resonance%%%. Upon irradiation (420-480 nm), the LOV2 C450A mutant protein gave an optical absorption spectrum characteristic of a flavin radical even in the absence of exogenous electron donors, thus demonstrating that the flavin mononucleotide (FMN) cofactor in its photogenerated triplet state is a potent oxidant for redox-active amino acid residues within the LOV2 domain. The FMN radical

in the LOV2 C450A mutant is N(5)-protonated, suggesting that the local pH close to the FMN is acidic enough so that the cysteine residue in the wild-type protein is likely to be also protonated. An electron paramagnetic %%%resonance%%% analysis of the photogenerated FMN radical gave information on the geometrical and electronic structure and the environment of the FMN cofactor. The experimentally determined hyperfine couplings of the FMN radical point to a highly restricted delocalization of the unpaired electron spin in the isoalloxazine moiety. In the light of these results a possible radical-pair mechanism for the formation of the FMN-C(4a)-cysteinyl adduct in LOV domains is discussed.

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17253989 BIOSIS NO.: 200300212708
Homodimerization of neuropeptide Y receptors investigated by fluorescence %%%resonance%%% energy transfer in living cells.
AUTHOR: Dinger Michaela C; Bader Juergen E; Kobor Andreas D; Kretzschmar Antje K; Beck-Sickinger Annette G (Reprint)
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JOURNAL: Journal of Biological Chemistry 278 (12): p10562-10571 March 21, 2003 2003
MEDIUM: print
ISSN: 0021-9258
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LANGUAGE: English

ABSTRACT: Up to now neuropeptide Y (NPY) receptors, which belong to the large family of G-protein-coupled receptors and are involved in a broad range of physiological processes, are believed to act as monomers. Studies with the Y1-receptor antagonist and Y4-receptor agonist GR231118, which binds with a 250-fold higher affinity than its monomer, led to the first speculation that NPY receptors can form homodimers. In the present work we used the fluorescence %%%resonance%%% energy transfer (FRET) to study homodimerization of the hY1-, hY2-, and hY5-receptors in living cells. For this purpose, we generated %%%fusion%%% proteins of NPY receptors and green fluorescent protein or spectral variants of green fluorescent protein (cyan, yellow, and %%%red%%% fluorescent protein), which can be used as FRET pairs. Two different FRET techniques, fluorescence microscopy and fluorescence spectroscopy, were applied. Both techniques clearly showed that the hY1-, hY2-, and hY5-NPY receptor subtypes are able to form homodimers. By using transiently transfected cells, as well as a stable cell line expressing the hY2-GFP %%%fusion%%% protein, we could demonstrate that the Y-GFP %%%fusion%%% proteins are still functional and that dimerization varies from 26 to 44% dependent on the receptor. However, homodimerization is influenced neither by NPY nor by Galpha protein binding.

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17253227 BIOSIS NO.: 200300211946
On the interaction between gp41 and membranes: The immunodominant loop stabilizes gp41 helical hairpin conformation.
AUTHOR: Peisajovich Sergio G; Blank Lior; Epand Raquel F; Epand Richard M; Shai Yechiel (Reprint)
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JOURNAL: Journal of Molecular Biology 326 (5): p1489-1501 7 March 2003 2003
MEDIUM: print
ISSN: 0022-2836 _(ISSN print)
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: gp41 is the protein responsible for the process of membrane %%%fusion%%% that allows primate lentiviruses (HIV and SIV) to enter into their host cells. gp41 ectodomain contains an N-terminal and a C-terminal heptad repeat region (NHR and CHR) connected by an immunodominant loop. In the absence of membranes, the NHR and CHR segments fold into a protease-resistant core with a trimeric helical hairpin structure. However, when the immunodominant loop is not present (either in a complex formed by HIV-1 gp41-derived NHR and CHR peptides or by mild treatment with protease of recombinant constructs of HIV-1 gp41 ectodomain, which also lack the N-terminal %%%fusion%%% peptide and the C-terminal Trp-rich region) membrane binding induces a conformational change in the gp41 core structure. Here, we further investigated whether covalently linking the NHR and CHR segments by the immunodominant loop affects this conformational change. Specifically, we analyzed a construct corresponding to a fragment of SIVmac239 gp41ectodomain (residues 27-149, named e-gp41) by means of surface plasmon %%%resonance%%%, Trp and %%%rhodamine%%% fluorescence, ATR-FTIR spectroscopy, and differential scanning calorimetry. Our results suggest that the presence of the loop stabilizes the trimeric helical hairpin both when e-gp41 is in aqueous solution and when it is bound to the membrane surface. Bearing in mind possible differences between HIV-1 and SIV gp41, and considering that the gp41 ectodomain constructs analyzed to date lack the N-terminal %%%fusion%%% peptide and the C-terminal Trp-rich region, we discuss our observations in relation to the mechanism of virus-induced membrane %%%fusion%%%.

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17245111 BIOSIS NO.: 200300203830
FRET-based in vivo screening for protein folding and increased protein stability.
AUTHOR: Philipps Bjorn; Hennecke Jens; Glockshuber Rudi (Reprint)
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JOURNAL: Journal of Molecular Biology 327 (1): p239-249 14 March, 2003

2003
MEDIUM: print
ISSN: 0022-2836 _(ISSN print)
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RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Fluorescence %%%resonance%%% energy transfer (FRET) was used to establish a novel in vivo screening system that allows rapid detection of protein folding and protein variants with increased thermodynamic stability in the cytoplasm of Escherichia coli. The system is based on the simultaneous %%%fusion%%% of the green fluorescent protein (GFP) to the C terminus of a protein X of interest, and of %%%blue%%%-fluorescent protein (BFP) to the N terminus of protein X. Efficient FRET from BFP to GFP in the ternary %%%fusion%%% protein is observed in vivo only when protein X is folded and brings BFP and GFP into close proximity, while FRET is lost when BFP and GFP are far apart due to unfolding or intracellular degradation of protein X. The screening system was validated by identification of antibody VL intradomains with increased thermodynamic stabilities from expression libraries after random mutagenesis, bacterial cell sorting, and colony screening.

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17184448 BIOSIS NO.: 200300143167
Mechanism Of Enhanced %%%Fusion%%% By A P296T Human Peripherin/rds Mutant.
AUTHOR: Krouse J C (Reprint); Stefano F P; Boesze-Battaglia K
AUTHOR ADDRESS: Molecular Biology, UMDNJ-SOM and GBSB-Stratford Division, Stratford, NJ, USA**USA
JOURNAL: ARVO Annual Meeting Abstract Search and Program Planner 2002 p Abstract No. 1396 2002 2002
MEDIUM: cd-rom
CONFERENCE/MEETING: Annual Meeting of the Association For Research in Vision and Ophthalmology Fort Lauderdale, Florida, USA May 05-10, 2002; 20020505
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Purpose: The C-terminus of peripherin/rds promotes membrane %%%fusion%%% through a %%%fusion%%% peptide domain, residues 311-325. A proline to threonine mutation upstream of the %%%fusion%%% peptide domain at position 296 results in enhanced %%%fusion%%%. The work presented herein address the mechanism by which this %%%fusion%%% enhancement occurs. Methods: A series of mutations of the proline at residue 296 were generated in the pGemex-2 vector containing a Not 1 cloned 1.1Kb fragment of human peripherin/rds cDNA. WT and mutant proteins were N-terminal tagged using a pcDNA 3.1 His B vector, allowing for antibody detection (Xpress epitope tag) and purification on a Ni+2column (polyhistidine tag) and transiently transfected into MDCK and COS-1 cells. Peripherin/rds-lipid recombinants were prepared using detergent dialysis with vesicles composed of PC:PS:cholesterol (4:4:1 mole ratio). %%%Fusion%%% was measured using %%%resonance%%% energy transfer techniques. Peripherin/rds localization was determined with specific

marker proteins using immunofluorescence microscopy. Results: The WT and P296A, P296L, P296E mutant peripherin/rds were expressed as 42 kDa monomers detected using western blot analysis with anti-Xpress antibody under denaturing conditions. The purified proteins were recombined into liposomes that fused with R18 ROS PM, confirming a viable functional assay for the proteins. WT-Xpress peripherin/rds showed cellular localization to both intracellular and plasma membranes. The P296T mutant initially localized to intracellular membranes, but upon confluence appeared mostly in the plasma membrane. Conclusions: These results suggest that an area upstream of the %%%fusion%%% peptide domain, analogous to SNARE-pin like regions in other %%%fusion%%% protein may aid in promoting the %%%fusion%%% competency of peripherin/rds. The C-terminus of peripherin/rds may provide the localization signal for incorporation of peripherin/rds into the plasma membrane of MDCK cells.

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17099249 BIOSIS NO.: 200300057968
Glycosylation of erythropoietin affects receptor binding kinetics: Role of electrostatic interactions.
AUTHOR: Darling Ryan J; Kuchibhotla Uma; Glaesner Wolfgang; Micanovic Radmila; Witcher Derrick R; Beals John M (Reprint)
AUTHOR ADDRESS: BioResearch Technologies and Proteins, Lilly Research Laboratories, Eli Lilly and Co., Indianapolis, IN, 46285, USA**USA
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JOURNAL: Biochemistry 41 (49): p14524-14531 December 10, 2002 2002
MEDIUM: print
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LANGUAGE: English

ABSTRACT: Erythropoietin (EPO) is a cytokine produced by the kidney whose function is to stimulate %%%red%%% blood cell production in the bone marrow. Previously, it was shown that the affinity of EPO for its receptor, EPOR, is inversely related to the sialylation of EPO carbohydrate. To better understand the properties of EPO that modulate its receptor affinity, various glycoforms were analyzed using surface plasmon %%%resonance%%%. The system used has been well characterized and is based on previous reports employing an EPOR-Fc %%%chimera%%% captured on a Protein A surface. Using three variants of EPO containing different levels of sialylation, we determined that sialic acid decreased the association rate constant (kon) about 3-fold. Furthermore, glycosylated EPO had a 20-fold slower kon than nonglycosylated EPO, indicating that the core carbohydrate also negatively impacted kon. The effect of electrostatic forces on EPO binding was studied by measuring binding kinetics in varying NaCl concentrations. Increasing NaCl concentration resulted in a slower kon while having little impact on koff, suggesting that long-range electrostatic interactions are primarily important in determining the rate of association between EPO and EPOR. Furthermore, the glycosylation content (i.e., nonglycosylated vs glycosylated, sialylated vs desialylated) affected the overall sensitivities of kon to (NaCl), indicating that sialic acid and the glycan itself each impact the overall effect of these electrostatic forces.

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17041552 BIOSIS NO.: 200300000271
Far-%%%red%%% fluorescent tag for protein labelling.
AUTHOR: Fradkov Arkady F; Verkhusha Vladislav V; Staroverov Dmitry B; Bulina Maria E; Yanushevich Yurii G; Martynov Vladimir I; Lukyanov Sergey ; Lukyanov Konstantin A (Reprint)
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JOURNAL: Biochemical Journal 368 (1): p17-21 15 November, 2002 2002
MEDIUM: print
ISSN: 0264-6021
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RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Practical applications of green fluorescent protein ('GFP')-like fluorescent proteins (FPs) from species of the class Anthozoa (sea anemones, corals and sea pens) are strongly restricted owing to their oligomeric nature. Here we suggest a strategy to overcome this problem by the use of two covalently linked identical %%%red%%% FPs as non-oligomerizing %%%fusion%%% tags. We have applied this approach to the dimeric far-%%%red%%% fluorescent protein HcRed1 and have demonstrated superiority of the tandem tag in the in vivo labelling of fine cytoskeletal structures and tiny nucleoli. In addition, a possibility of effective fluorescence %%%resonance%%% energy transfer ('FRET') between enhanced yellow FP mutant ('EYFP') and tandem HcRed1 was demonstrated in a protease assay.

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17020940 BIOSIS NO.: 200200614451
Ca2+-dependent and phospholipid-independent binding of annexin 2 and annexin 5
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AUTHOR ADDRESS: R and D Department, Canadian Blood Services, University of British Columbia, 2211 Wesbrook Mall, Vancouver, BC, V6T 2B5, Canada** Canada
JOURNAL: Biochemical Journal 367 (3): p895-900 1 November, 2002 2002
MEDIUM: print
ISSN: 0264-6021
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RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Annexins are a family of homologous proteins that associate with anionic phospholipid (aPL) in the presence of Ca2+. Evidence that the

function of one annexin type may be regulated by another was recently reported in studies investigating cytomegalovirus-aPL interactions, where the fusogenic function of annexin 2 (A2) was attenuated by annexin 5 (A5). This observation suggested that A2 may bind directly to A5. In the present study, we demonstrated this interaction. The A2-A5 complex was first detected utilizing (covalently linked) %%%fluorescein%%%-labelled A5 (F-A5) as a reporter group. The interaction required concentrations of Ca2+ in the millimolar range, had an apparent dissociation constant (Kd(app)) of 1 nM at 2 mM Ca2+ and was independent of aPL. A2 bound comparably with F-A5 pre-equilibrated with an amount of aPL that could bind just the F-A5 or to an excess amount of aPL providing sufficient binding sites for all of F-A5 and A2. A2-A5 complex formation was corroborated in an experiment, where (125I)A2 associated in a Ca2+-dependent manner with A5 coated on to polystyrene. Surface plasmon %%%resonance%%% was used as a third independent method to demonstrate the binding of A2 and A5 and, furthermore, supported the conclusion that the monomeric and tetrameric forms of A2 bind equivalently to A5. Together these results demonstrate an A2-A5 interaction and provide an explanation as to how A5 inhibits the previously reported A2-dependent enhancement of virus-aPL %%%fusion%%%.

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16905378 BIOSIS NO.: 200200498889
Spectrally resolved fluorescence lifetime imaging microscopy
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JOURNAL: Applied Spectroscopy 56 (2): p155-166 February, 2002 2002
MEDIUM: print
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LANGUAGE: English

ABSTRACT: We report a system for collecting spectrally resolved fluorescent lifetime images. Frequency domain fluorescence lifetime detection was combined with two-dimensional spectral imaging in a programmable array microscope. The spectroscopic fluorescence lifetime imaging microscopy (sFLIM) system has a resolution of apprx50 (lambda/DELTAlambda) in the current arrangement and a wavelength range of apprx430-750 nm. With the sFLIM system, we recorded the lifetime spectra of %%%rhodamine%%% 6G, %%%rhodamine%%% B, and the DNA intercalation dye propidium iodide (PI) in cuvettes and an EGFP-%%%fusion%%% of the histone 2A variant D protein in Drosophila salivary gland explants in the presence and absence of PI. In the absence of PI, the EGFP-%%%fusion%%% exhibited a lifetime of 2.7 ns with little variation in wavelength. The lifetime of PI alone ranged from apprx1 ns in buffer to apprx18 ns when intercalated in the nuclei of intact cells. The combination of EGFP and PI in the Drosophila salivary gland explants exhibited strong fluorescence %%%resonance%%% energy transfer (FRET), a result consistent with the known nucleosomal structure of eukaryotic chromatin.

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16810855 BIOSIS NO.: 200200404366
Bioluminescence %%%resonance%%% energy transfer (BRET). A tool to study protein-protein interactions in Arabidopsis
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JOURNAL: Plant Physiology (Rockville) 129 (2): p430-431 June, 2002 2002
MEDIUM: print
ISSN: 0032-0889
DOCUMENT TYPE: Article
RECORD TYPE: Citation
LANGUAGE: English

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16792027 BIOSIS NO.: 200200385538
The cytoplasmic tail of invariant chain regulates endosome %%%fusion%%% and morphology
AUTHOR: Nordeng Tommy W; Gregers Tone F; Kongsvik Thomas Lasker; Meresse Stephane; Gorvel Jean-Pierre; Jourdan Fabrice; Motta Andrea; Bakke Oddmund (Reprint)
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JOURNAL: Molecular Biology of the Cell 13 (6): p1846-1856 June, 2002 2002
MEDIUM: print
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ABSTRACT: The major histocompatibility complex class II associated invariant chain (Ii) has been shown to inhibit endocytic transport and to increase the size of endosomes. We have recently found that this property has a significant impact on antigen processing and presentation. Here, we show in a cell-free endosome %%%fusion%%% assay that expression of Ii can increase %%%fusion%%% after phosphatidylinositol 3-kinase activity is blocked by wortmannin. In live cells wortmannin was also not able to block formation of the Ii-induced enlarged endosomes. The effects of Ii on endosomal transport and morphology depend on elements within the cytoplasmic tail. Data from mutagenesis analysis and nuclear magnetic %%%resonance%%%-based structure calculations of the Ii cytoplasmic tail demonstrate that free negative charges that are not involved in internal salt bridges are essential for both interactions between the tails and for the formation of enlarged endosomes. This correlation indicates that it is interactions between the Ii cytoplasmic tails that are involved in endosome %%%fusion%%%. The combined data from live cells, cell-free assays, and molecular dynamic simulations suggest that Ii molecules on different vesicles can promote endosome docking and %%%fusion%%% and thereby control endosomal traffic of membrane proteins and endosomal content.

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16734456 BIOSIS NO.: 200200327967
Fluorescent probes for monitoring virus %%%fusion%%% kinetics: Comparative evaluation of reliability
AUTHOR: Nunes-Correia Isabel; Eulalio Ana; Nir Shlomo; Duzgunes Nejat; Ramalho-Santos Joao; Pedroso de Lima Maria C (Reprint)
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JOURNAL: Biochimica et Biophysica Acta 1561 (1): p65-75 19 March, 2002 2002
MEDIUM: print
ISSN: 0006-3002
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RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Fluorescence assays for viral membrane %%%fusion%%% employ lipidic probes whose kinetics of fluorescence dequenching should mimic the actual kinetics of membrane merging. We examined the %%%fusion%%% of influenza virus with CEM cells, erythrocyte ghosts or liposomes by monitoring the fluorescence dequenching of each one of the three probes, octadecylrhodamine B chloride (R18), N-(lissamine %%%rhodamine%%% B sulfonyl)phosphatidylethanolamine (Rh-PE), or rac-2,3-dioleoylglycerol ester of %%%rhodamine%%% B (DORh-B), inserted into the virus membrane. Experimental conditions were designed to allow a clear distinction between membrane mixing and non-specific probe transfer. Fluorescence dequenching observed with Rh-PE was much slower than with R18, unless a particular experimental procedure was used. Using liposomes as a target membrane, the kinetics and extent of the decrease in %%%resonance%%% energy transfer between N-(7-nitro-2,1,3-benzoxadiazol-4-yl)phosphatidylethanolamine (NBD-PE) and Rh-PE, initially embedded in the liposome membrane, were matched by that of the dequenching of viral R18, but not of viral Rh-PE. DORh-B was found not to be appropriate to follow membrane merging. Our results indicate that on a time scale of several minutes R18 more accurately reflects the kinetics of membrane %%%fusion%%%. Nevertheless, control experiments should be performed to evaluate non-specific probe transfer of R18 molecules, whose contribution to fluorescence dequenching can become significant after long incubation times.

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16734016 BIOSIS NO.: 200200327527
Targetability and intracellular delivery of anti-BCG antibody-modified, pH-sensitive fusogenic immunoliposomes to tumor cells
AUTHOR: Mizoue Toshiro; Horibe Toshiya; Maruyama Kazuo (Reprint); Takizawa Tomoko; Iwatsuru Motoharu; Kono Kenji; Yanagie Hironobu; Moriyasu Fuminori

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JOURNAL: International Journal of Pharmaceutics (Kidlington) 237 (1-2): p 129-137 26 April, 2002 2002
MEDIUM: print
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RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: We prepared tumor-specific immunoliposomes by coupling anti-BCG monoclonal antibodies to pH-sensitive fusogenic liposomes modified with succinylated polyglycidol (sucPG), in order to obtain efficient binding to, and endocytotic internalization into, the tumor cells. Mouse colon carcinoma 26 cells, which are known to share a common antigen with BCG, were used in in vitro experiments. BCG-sucPG immunoliposomes showed %%%fusion%%% ability under acidic conditions. Fluorescence microscopic observation indicated that BCG-sucPG immunoliposomes bound to colon 26 tumor cells and induced receptor-mediated endocytosis at 37 degreeC. %%%Fusion%%% assay by %%%resonance%%% energy transfer using N-(7-nitro-2-1,3-benzoxadiazol-4-yl) phosphatidylethanolamine and N-(lissamine %%%rhodamine%%% B sulfonyl) diacyl phosphatidylethanolamine suggested that %%%fusion%%% between BCG-sucPG immunoliposomes and endosomal and/or lysozomal membrane did occur. These results imply that the BCG-sucPG immunoliposomes transfer their content into the cytoplasm by fusing with the endosomal and/or lysozomal membrane after recognition of target cells and subsequent internalization into the cells by endocytosis.

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16719777 BIOSIS NO.: 200200313288
The BRET2/arrestin assay: An assay of choice to study G protein-coupled receptor (GPCR) activation in stably transfected cells
AUTHOR: Bertrand Lucie (Reprint); Parent Stephane (Reprint); Caron Mireille (Reprint); Legault Mireille (Reprint); Joly Erik (Reprint); Angers Stephane; Bouvier Michel; Houle Benoit (Reprint); Menard Luc (Reprint)
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JOURNAL: FASEB Journal 16 (4): pA196 March 20, 2002 2002
MEDIUM: print
CONFERENCE/MEETING: Annual Meeting of the Professional Research Scientists on Experimental Biology New Orleans, Louisiana, USA April 20-24, 2002; 20020420
ISSN: 0892-6638
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LANGUAGE: English

ABSTRACT: BRET2 (Bioluminescence %%%Resonance%%% Energy Transfer) allows monitoring energy transfer between the donor Renilla luciferase (Rluc) and the acceptor Green Fluorescent Protein (GFP2), in the presence of the cell permeable substrate DeepBlueCTM. If the GFP is brought into close proximity to Rluc, it will absorb part of the 395 nm light energy and

reemit at 510 nm. BRET2 signals are therefore easily determined by measuring the ratio of green over %%%blue%%% light (510/395 nm). Using BRET2, we developed a generic GPCR assay based on the observation that activation of the majority of GPCRs by agonists leads to the interaction of the receptor with beta-arrestin. We established a cell line stably expressing the GFP2:beta-arrestin2 %%%fusion%%% protein from which we derived a double stable line expressing the vasopressin 2 receptor fused to Rluc. Using this double stable line, we carried out the pharmacological characterization of agonists/antagonists in BRET2/arrestin assays and showed that the pharmacology was similar to what was observed with standard assays. Overall, our data show that BRET2/arrestin assay using recombinant cell lines can be used as a tool to detect modulation of GPCR activity by ligands, which could be used for compound characterization.

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16661242 BIOSIS NO.: 200200254753
Hydrostatic pressure induces the %%%fusion%%%-active state of enveloped viruses
AUTHOR: Gaspar Luciane P; Silva Ana C B; Gomes Andre M O; Freitas Monica S; Ano Bom Ana P D; Schwarcz Waleska D; Mestecky Jiri; Novak Miroslav J; Foguel Debora; Silva Jerson L (Reprint)
AUTHOR ADDRESS: Programa de Biologia Estrutural, Departamento de Bioquimica Medica, Centro Nacional de Ressonancia Magnetica Nuclear de Macromoleculas, Instituto de Ciencias Biomedicas, Universidade Federal do Rio de Janeiro, 21941-590, Rio de Janeiro, RJ, Brazil**Brazil
JOURNAL: Journal of Biological Chemistry 277 (10): p8433-8439 March 8, 2002 2002
MEDIUM: print
ISSN: 0021-9258
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RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Enveloped animal viruses must undergo membrane %%%fusion%%% to deliver their genome into the host cell. We demonstrate that high pressure inactivates two membrane-enveloped viruses, influenza and Sindbis, by trapping the particles in a %%%fusion%%%-intermediate state. The pressure-induced conformational changes in Sindbis and influenza viruses were followed using intrinsic and extrinsic fluorescence spectroscopy, circular dichroism, and %%%fusion%%%, plaque, and hemagglutination assays. Influenza virus subjected to pressure exposes hydrophobic domains as determined by tryptophan fluorescence and by the binding of bis-8-anilino-1-naphthalenesulfonate, a well established marker of the fusogenic state in influenza virus. Pressure also produced an increase in the %%%fusion%%% activity at neutral pH as monitored by fluorescence %%%resonance%%% energy transfer using lipid vesicles labeled with fluorescence probes. Sindbis virus also underwent conformational changes induced by pressure similar to those in influenza virus, and the increase in %%%fusion%%% activity was followed by pyrene excimer fluorescence of the metabolically labeled virus particles. Overall we show that pressure elicits subtle changes in the whole structure of the enveloped viruses triggering a conformational change that is similar to

the change triggered by low pH. Our data strengthen the hypothesis that the native conformation of %%%fusion%%% proteins is metastable, and a cycle of pressure leads to a final state, the %%%fusion%%%-active state, of smaller volume.

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16592024 BIOSIS NO.: 200200185535
Allele-specific in situ analysis of microchimerism by fluorescence %%%resonance%%% energy transfer (FRET) in nonhuman primate tissues
AUTHOR: Lobashevsky A L; Jiang X L; Thomas J M (Reprint)
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JOURNAL: Human Immunology 63 (2): p108-120 February, 2002 2002
MEDIUM: print
ISSN: 0198-8859
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RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Rhesus monkeys are relevant models for tolerance induction. Hematopoetic %%%chimerism%%% is believed to be one of these strategies. The purpose of this study was to detect donor class I A locus allele specific mRNA in Rhesus monkey kidney recipient. We report here for the first time the results of frequency %%%resonance%%% energy transfer (FRET) hybridization technology in frozen tissues. Frequency %%%resonance%%% energy transfer hybridization was performed by using two Mamu-A*05 allele specific oligonucleotides: a donor probe labeled with FITC and acceptor probe conjugated to Texas %%%Red%%%. The PCR-SSP microchimerism analysis method produced 0.05% and 0.5% of donor DNA for Mamu-DRB1*1002 and Mamu-DRBw301/3 alleles, respectively. The donor cells were detected in mesenteric and/or inguinal lymph nodes, spleen, and liver, where the signal was the strongest. The results of FRET hybridization demonstrated the identical staining pattern in the recipient frozen tissues to that determined by PCR-SSP. Following FRET hybridization, the sections underwent immunohistochemical analysis, which revealed that donor cells had CD8+ phenotype. We demonstrate here for the first time that FRET in situ hybridization technique can be utilized for microchimerism analysis in frozen tissues. We conclude that using two donor mRNA specific oligonucleotide probes, rather than one, produce higher specificity.

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16557611 BIOSIS NO.: 200200151122
Interaction of the heart-specific LIM domain protein, FHL2, with DNA-binding nuclear protein, hNP220
AUTHOR: Ng Enders Kai On; Chan Kwok Keung; Wong Chi Hang; Tsui Stephen Kwok Wing; Ngai Sai Ming; Lee Simon Ming Yuen; Kotaka Masayo; Lee Cheuk Yu; Waye Mary Miu Yee; Fung Kwok Pui (Reprint)

AUTHOR ADDRESS: Department of Biochemistry, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong SAR, China**China
JOURNAL: Journal of Cellular Biochemistry 84 (3): p556-566 2001 2001
MEDIUM: print
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RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Using a yeast two-hybrid library screen, we have identified that the heart specific FHL2 protein, four-and-a-half LIM protein 2, interacted with human DNA-binding nuclear protein, hNP220. Domain studies by the yeast two-hybrid interaction assay revealed that the second LIM domain together with the third and the fourth LIM domains of FHL2 were responsible to the binding with hNP220. Using green fluorescent protein (GFP)-FHL2 and %%%blue%%% fluorescent protein (BFP)-hNP220 %%%fusion%%% proteins co-expressed in the same cell, we demonstrated a direct interaction between FHL2 and hNP220 in individual nucleus by two-%%%fusion%%% Fluorescence %%%Resonance%%% Energy Transfer (FRET) assay. Besides, Western blot analysis using affinity-purified anti-FHL2 antipeptide antibodies confirmed a 32-kDa protein of FHL2 in heart only. Virtually no expression of FHL2 protein was detected in brain, liver, lung, kidney, testis, skeletal muscle, and spleen. Moreover, the expression of FHL2 protein was also detectable in the human diseased heart tissues. Our results imply that FHL2 protein can shuttle between cytoplasm and nucleus and may act as a molecular adapter to form a multicomplex with hNP220 in the nucleus, thus we speculate that FHL2 may be particularly important for heart muscle differentiation and the maintenance of the heart phenotype.

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16525669 BIOSIS NO.: 200200119180
Membrane-active properties of alpha-MSH analogs: Aggregation and %%%fusion%%% of liposomes triggered by surface-conjugated peptides
AUTHOR: Lima de Souza Debora; Frisch Benoit; Duportail Guy; Schuber Francis (Reprint)
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JOURNAL: Biochimica et Biophysica Acta 1558 (2): p222-237 1 February, 2002 2002
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RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Reaction of the melanotropin hormone analogs (Nle4,D-Phe7)-alpha-MSH and (Nle4,D-Phe7)-alpha-MSH(4-10), which were extended at their N-terminus by a thiol-functionalized spacer arm, with preformed liposomes containing thiol-reactive (phospho)lipid derivatives resulted in the aggregation of the vesicles and in a partial leakage of their inner contents. This aggregation/leakage effect, which was only

observed when the peptides were covalently conjugated to the surface of the liposomes, was correlated with the %%%fusion%%% of the vesicles as demonstrated by the observed decrease in %%%resonance%%% energy transfer between probes in a membrane lipid mixing assay. A limited %%%fusion%%% was confirmed by monitoring the mixing of the liposome inner contents (formation of 1-aminonaphthalene-3,6,8-trisulfonic acid/p-xylene bis(pyridinium bromide) complex). The membrane-active properties of the peptides could be correlated with changes in the fluorescence emission spectra of their tryptophan residue, which suggested that after their covalent binding to the outer surface of the liposomes they can partition within the core of the bilayers. A %%%blue%%% shift of 10 nm was observed for (Nle4,D-Phe7)-alpha-MSH which was correlated with an increase in fluorescence anisotropy and with changes in the accessibility of the coupled peptide as assessed by the quenching of fluorescence of its tryptophan residue by iodide (Stern-Volmer plots). These results should be related to the previously described capacity of alpha-MSH, and analogs, to interact with membranes and with the favored conformation of these peptides which, via a beta-turn, segregate their central hydrophobic residues into a domain that could insert into membranes and, as shown here, trigger their destabilization.

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16416229 BIOSIS NO.: 200200009740
Detection of epitope-tagged proteins in flow cytometry: Fluorescence %%%resonance%%% energy transfer-based assays on beads with femtomole resolution
AUTHOR: Buranda Tione (Reprint); Lopez Gabriel P (Reprint); Simons Peter; Pastuszyn Andrzej; Sklar Larry A (Reprint)
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JOURNAL: Analytical Biochemistry 298 (2): p151-162 November 15, 2001 2001
MEDIUM: print
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LANGUAGE: English

ABSTRACT: Epitope tagging of expressed proteins is a versatile tool for the detection and purification of the proteins. This approach has been used in protein-protein interaction studies, protein localization, and immunoprecipitation. Among the most popular tag systems is the FLAG epitope tag, which is recognized by three monoclonal antibodies M1, M2, and M5. We describe novel approaches to the detection of epitope-tagged proteins via fluorescence %%%resonance%%% energy transfer on beads. We have synthesized and characterized biotinylated and %%%fluorescein%%% -labeled FLAG peptides and examined the binding of FLAG peptides to commercial streptavidin beads using flow cytometric analysis. A requirement of assay development is the elucidation of parameters that characterize the binding interactions between component systems. We have thus compiled a set of Kd values determined from a series of equilibrium binding experiments with beads, peptides, and antibodies. We have defined conditions for binding biotinylated and %%%fluoresceinated%%% FLAG peptides to beads. Site occupancies of the peptides were determined to be

on the order of several million sites per bead and Kd values in the 0.3-2.0 nM range. The affinity for antibody attachment to peptides was determined to be in the low nanomolar range (less than 10 nM) for measurements on beads and solution. We demonstrate the applicability of this methodology to assay development, by detecting femtomole amounts of N-terminal FLAG-bacteria alkaline phosphatase %%%fusion%%% protein. These characterizations form the basis of generalizable and high throughput assays for proteins with known epitopes, for research, proteomic, or clinical applications.

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16305748 BIOSIS NO.: 200100477587
Fluorescence %%%resonance%%% energy transfer microscopy of localized protein interactions in the living cell nucleus
AUTHOR: Day Richard N (Reprint); Periasamy Ammasi; Schaufele Fred
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JOURNAL: Methods (Orlando) 25 (1): p4-18 September, 2001 2001
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RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Cells respond to environmental cues by modifying protein complexes in the nucleus to produce a change in the pattern of gene expression. In this article, we review techniques that allow us to visualize these protein interactions as they occur in living cells. The cloning of genes from marine organisms that encode fluorescent proteins provides a way to tag and monitor the intracellular behavior of expressed %%%fusion%%% proteins. The genetic engineering of jellyfish green fluorescent protein (GFP) and the recent cloning of a sea anemone %%%red%%% fluorescent protein (RFP) have provided fluorescent tags that emit light at wavelengths ranging from the %%%blue%%% to the %%%red%%% spectrum. Several of these color variants can be readily distinguished by fluorescence microscopy, allowing them to be used in combination to monitor the behavior of two or more independent proteins in the same living cell. We describe the use of this approach to examine where transcription factors are assembled in the nucleus. To demonstrate that these labeled nuclear proteins are interacting, however, requires spatial resolution that exceeds the optical limit of the light microscope. This degree of spatial resolution can be achieved with the conventional light microscope using the technique of fluorescence %%%resonance%%% energy transfer (FRET). The application of FRET microscopy to detect the interactions between proteins labeled with the color variants of GFP and the limitations of the FRET approach are discussed. The use of different-color fluorescent proteins in combination with FRET offers the opportunity to study the complex behavior of key regulatory proteins in their natural environment within the living cell.

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16243526 BIOSIS NO.: 200100415365
Optimal inhibition of X4 HIV isolates by the CXC chemokine stromal cell-derived factor 1alpha requires interaction with cell surface heparan sulfate proteoglycans
AUTHOR: Valenzuela-Fernandez Agustin; Palanche Tania; Amara Ali; Magerus Aude; Altmeyer Ralf; Delaunay Thierry; Virelizier Jean-Louis; Baleux Francoise; Galzi Jean-Luc; Arenzana-Seisdedos Fernando (Reprint)
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JOURNAL: Journal of Biological Chemistry 276 (28): p26550-26558 July 13, 2001 2001
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LANGUAGE: English

ABSTRACT: The chemokine stromal cell-derived factor 1 (SDF-1) is the natural ligand for CXC chemokine receptor 4 (CXCR4). SDF-1 inhibits infection of CD4+ cells by X4 (CXCR4-dependent) human immunodeficiency virus (HIV) strains. We previously showed that SDF-1alpha interacts specifically with heparin or heparan sulfates (HSs). Herein, we delimited the boundaries of the HS-binding domain located in the first beta-strand of SDF-1alpha as the critical residues. We also provide evidence that binding to cell surface heparan sulfate proteoglycans (HSPGs) determines the capacity of SDF-1alpha to prevent the fusogenic activity of HIV-1 X4 isolates in leukocytes. Indeed, SDF-1alpha mutants lacking the capacity to interact with HSPGs showed a substantially reduced capacity to prevent cell-to-cell %%%fusion%%% mediated by X4 HIV envelope glycoproteins. Moreover, the enzymatic removal of cell surface HS diminishes the HIV-inhibitory capacity of the chemokine to the levels shown by the HS-binding-disabled mutant counterparts. The mechanisms underlying the optimal HIV-inhibitory activity of SDF-1alpha when attached to HSPGs were investigated. Combining fluorescence %%%resonance%%% energy transfer and laser confocal microscopy, we demonstrate the concomitant binding of SDF-1alpha to CXCR4 and HSPGs at the cell membrane. Using FRET between a Texas %%%Red%%%-labeled SDF-1alpha and an enhanced green fluorescent protein-tagged CXCR4, we show that binding of SDF-1alpha to cell surface HSPGs modifies neither the kinetics of occupancy nor activation in real time of CXCR4 by the chemokine. Moreover, attachment to HSPGs does not modify the potency of the chemokine to promote internalization of CXCR4. Attachment to cellular HSPGs may co-operate in the optimal anti-HIV activity of SDF-1alpha by increasing the local concentration of the chemokine in the surrounding environment of CXCR4, thus facilitating sustained occupancy and down-regulation of the HIV coreceptor.

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16223994 BIOSIS NO.: 200100395833
The rate of lipid transfer during %%%fusion%%% depends on the structure of

fluorescent lipid probes: A new chain-labeled lipid transfer probe pair
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JOURNAL: Biochemistry 40 (28): p8292-8299 July 17, 2001 2001
MEDIUM: print
ISSN: 0006-2960
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RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: A number of fluorescent probes have been used to follow membrane %%%fusion%%% events, particularly intermixing of lipids. None of them is ideal. The most popular pair of probes is NBD-PE and Rh-PE, in which the fluorescent groups are attached to the lipid headgroups, making them sensitive to changes in the surrounding medium. Here we present a new assay for monitoring lipid transfer during membrane %%%fusion%%% using the acyl chain tagged fluorescent probes BODIPY500-PC and BODIPY530-PE. Like the NBD-PE/Rh-PE assay, this assay is based on fluorescence %%%resonance%%% energy transfer (FRET) between the donor, BODIPY500, and the acceptor, BODIPY530. The magnitude of FRET is sensitive to the probe surface concentration, allowing one to detect movement of probes from labeled to unlabeled vesicles during %%%fusion%%%. The high quantum yield of fluorescence, high efficiency of FRET (Ro is estimated to be apprx60 ANG), photostability, and localization in the central hydrophobic region of a bilayer all make this pair of probes quite promising for detecting %%%fusion%%%. We have compared this and two other lipid mixing assays for their abilities to detect the initial events of poly(ethylene glycol) (PEG)-mediated %%%fusion%%% of small unilamellar vesicles (SUVs). We found that the BODIPY500/530 assay showed lipid transfer rates consistent with those obtained using the DPHpPC self-quenching assay, while lipid mixing rates measured with the NBD-PE/Rh-PE RET assay were significantly slower. We speculate that the bulky labeled headgroups of NBD-PE and especially Rh-PE molecules hamper movement of probes through the stalk between fusing vesicles, and thus reduce the apparent rate of lipid mixing.

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16177721 BIOSIS NO.: 200100349560
Multiphoton-evoked color change of DsRed as an optical highlighter for cellular and subcellular labeling
AUTHOR: Marchant Jonathan S; Stutzmann Grace E; Leissring Malcolm A; LaFerla Frank M; Parker Ian (Reprint)
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JOURNAL: Nature Biotechnology 19 (7): p645-649 July, 2001 2001
MEDIUM: print
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ABSTRACT: DsRed, a recently cloned %%%red%%% fluorescent protein, has attracted great interest as an expression tracer and %%%fusion%%% partner for multicolor imaging. We report that three-photon excitation (lambda<760 nm) rapidly changes the fluorescence of DsRed from %%%red%%% to green when viewed subsequently by conventional (one-photon) epifluorescence. Mechanistically, three-photon excitation (lambda<760 nm) selectively bleaches the mature, %%%red%%%-emitting form of DsRed, thereby enhancing emission from the immature green form through reduction of fluorescence %%%resonance%%% energy transfer (FRET). The "greening" effect occurs in live mammalian cells at the cellular and subcellular levels, and the resultant color change persists for >30 h without affecting cell viability. This technique allows individual cells, organelles, and %%%fusion%%% proteins to be optically marked and has potential utility for studying cell lineage, organelle dynamics, and protein trafficking, as well as for selective retrieval of cells from a population. We describe optimal parameters to induce the color change of DsRed, and demonstrate applications that show the potential of this optical highlighter.

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16147123 BIOSIS NO.: 200100318962
Expression of fluorescently tagged connexins: A novel approach to rescue function of oligomeric DsRed-tagged proteins
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JOURNAL: FEBS Letters 498 (1): p11-15 1 June, 2001 2001
MEDIUM: print
ISSN: 0014-5793
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LANGUAGE: English

ABSTRACT: A novel, brilliantly %%%red%%% fluorescent protein, DsRed has become available recently opening up a wide variety of experimental opportunities for double labeling and fluorescence %%%resonance%%% electron transfer experiments in combination with green fluorescent protein (GFP). Unlike in the case of GFP, proteins tagged with DsRed were often found to aggregate within the cell. Here we report a simple method that allows rescuing the function of an oligomeric protein tagged with DsRed. We demonstrate the feasibility of this approach on the subunit proteins of an oligomeric membrane channel, gap junction connexins. Additionally, DsRed fluorescence was easily detected 12-16 h post transfection, much earlier than previously reported, and could readily be differentiated from co-expressed GFP. Thus, this approach can eliminate the major drawbacks of this highly attractive autofluorescent protein.

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16021070 BIOSIS NO.: 200100192909
Integrated volume visualization of functional image data and anatomical surfaces using normal %%%fusion%%%
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JOURNAL: Human Brain Mapping 12 (4): p203-218 April, 2001 2001
MEDIUM: print
ISSN: 1065-9471
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RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: A generic method, called normal %%%fusion%%%, for integrated three-dimensional (3D) visualization of functional data with surfaces extracted from anatomical image data is described. The first part of the normal %%%fusion%%% method derives quantitative values from functional input data by sampling the latter along a path determined by the (inward) normal of a surface extracted from anatomical data; the functional information is thereby projected onto the anatomical surface independently of the viewpoint. %%%Fusion%%% of the anatomical and functional information is then performed with a color-encoding scheme based on the HSV model. This model is preferred over the RGB model to allow easy, rapid, and intuitive retrospective manipulation of the color encoding of the functional information in the integrated display, and two possible strategies for this manipulation are explained. The results first show several clinical examples that are used to demonstrate the viability of the normal %%%fusion%%% method. These same examples are then used to evaluate the two HSV color manipulation strategies. Furthermore, five nuclear medicine physicians used several other clinical cases to evaluate the overall approach for manipulation of the color encoded functional contribution to an integrated 3D visualization. The integrated display using the normal %%%fusion%%% technique combined with the added functionality provided by the retrospective color manipulation was highly appreciated by the clinicians and can be considered an important asset in the investigation of data from multiple modalities.

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15991764 BIOSIS NO.: 200100163603
%%%Red%%% fluorescent protein from Discosoma as a %%%fusion%%% tag and a partner for fluorescence %%%resonance%%% energy transfer
AUTHOR: Mizuno Hideaki; Sawano Asako; Eli Pharhad; Hama Hiroshi; Miyawaki Atsushi (Reprint)
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JOURNAL: Biochemistry 40 (8): p2502-2510 February 27, 2001 2001
MEDIUM: print
ISSN: 0006-2960
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LANGUAGE: English

ABSTRACT: The biochemical and biophysical properties of a %%%red%%% fluorescent protein from a Discosoma species (DsRed) were investigated. The recombinant DsRed expressed in E. coli showed a complex absorption spectrum that peaked at 277, 335, 487, 530, and 558 nm. Excitation at each of the absorption peaks produced a main emission peak at 583 nm, whereas a subsidiary emission peak at 500 nm appeared with excitation only at 277 or 487 nm. Incubation of E. coli or the protein at 37 degreeC facilitated the maturation of DsRed, resulting in the loss of the 500-nm peak and the enhancement of the 583-nm peak. In contrast, the 500-nm peak predominated in a mutant DsRed containing two amino acid substitutions (Y120H/K168R). Light-scattering analysis revealed that DsRed proteins expressed in E. coli and HeLa cells form a stable tetramer complex. DsRed in HeLa cells grown at 37 degreeC emitted predominantly at 583 nm. The %%%red%%% fluorescence was imaged using a two-photon laser (Nd:YLF, 1047 nm) as well as a one-photon laser (He:Ne, 543.5 nm). When fused to calmodulin, the %%%red%%% fluorescence produced an aggregation pattern only in the cytosol, which does not reflect the distribution of calmodulin. Despite the above spectral and structural complexity, fluorescence %%%resonance%%% energy transfer (FRET) between Aequorea green fluorescent protein (GFP) variants and DsRed was achieved. Dynamic changes in cytosolic free Ca2+ concentrations were observed with %%%red%%% cameleons containing yellow fluorescent protein (YFP), cyan fluorescent protein (CFP), or Sapphire as the donor and RFP as the acceptor, using conventional microscopy and one- or two-photon excitation laser scanning microscopy. Particularly, the use of the Sapphire-DsRed pair rendered the %%%red%%% cameleon tolerant of acidosis occurring in hippocampal neurons, because both Sapphire and DsRed are extremely pH-resistant.

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15985073 BIOSIS NO.: 200100156912
Dissection of central carbon metabolism of hemoglobin-expressing Escherichia coli by 13C nuclear magnetic %%%resonance%%% flux distribution analysis in microaerobic bioprocesses
AUTHOR: Frey Alexander D; Fiaux Jocelyne; Szyperski Thomas; Wuthrich Kurt; Bailey James E; Kallio Pauli T (Reprint)
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JOURNAL: Applied and Environmental Microbiology 67 (2): p680-687 February, 2001 2001
MEDIUM: print
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LANGUAGE: English

ABSTRACT: Escherichia coli MG1655 cells expressing Vitreoscilla hemoglobin (VHb), Alcaligenes eutrophus flavohemoprotein (FHP), the N-terminal hemoglobin domain of FHP (FHPg), and a %%%fusion%%% protein which comprises VHb and the A. eutrophus C-terminal reductase domain (VHb-%%%Red%%%) were grown in a microaerobic bioreactor to study the effects

of low oxygen concentrations on the central carbon metabolism, using fractional 13C-labeling of the proteinogenic amino acids and two-dimensional (13C, 1H)-correlation nuclear magnetic %%%resonance%%% (NMR) spectroscopy. The NMR data revealed differences in the intracellular carbon fluxes between E. coli cells expressing either VHb or VHb-%%%Red%%% and cells expressing A. eutrophus FHP or the truncated heme domain (FHPg). E. coli MG1655 cells expressing either VHb or VHb-%%%Red%%% were found to function with a branched tricarboxylic acid (TCA) cycle. Furthermore, cellular demands for ATP and reduction equivalents in VHb- and VHb-%%%Red%%%-expressing cells were met by an increased flux through glycolysis. In contrast, in E. coli cells expressing A. eutrophus hemeproteins, the TCA cycle is running cyclically, indicating a shift towards a more aerobic regulation. Consistently, E. coli cells displaying FHP and FHPg activity showed lower production of the typical anaerobic by-products formate, acetate, and D-lactate. The implications of these observations for biotechnological applications are discussed.

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15948107 BIOSIS NO.: 200100119946
Detection of the swings of the lever arm of a myosin motor by fluorescence %%%resonance%%% energy transfer of green and %%%blue%%% fluorescent proteins
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JOURNAL: Methods (Orlando) 22 (4): p355-363 December, 2000 2000
MEDIUM: print
ISSN: 1046-2023
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RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The "lever-arm" model of a myosin motor predicts that the lever-arm domain in the myosin head tilts and swings against the catalytic domain during ATP hydrolysis, resulting in force generation. To investigate if this "swing" of the lever arm really occurs during the hydrolysis of ATP, we employed fluorescence %%%resonance%%% energy transfer (FRET) between two fluorescent proteins (green (GFP) and %%%blue%%% (BFP)) fused to the N and C termini of the Dictyostelium myosin-motor domain. FRET measurements showed that the C-terminal BFP in the %%%fusion%%% protein first swings against the N-terminal GFP at the isomerization step of the ATP hydrolysis cycle and then swings back at the phosphate-release step. Because the C-terminal BFP mimics the motion of the lever arm, the result indicates that the lever arm swings at the specific steps of the ATP hydrolysis cycle, i.e., at the isomerization and phosphate-release steps. The latter swing may correspond to the power stroke of myosin, while the former may be related to the recovery stroke.

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15947306 BIOSIS NO.: 200100119145
Hypertrophy of the posterior longitudinal ligament is a prodromal condition to ossification: A cervical myelopathy case report
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JOURNAL: Spine 26 (1): p110-114 January 1, 2001 2001
MEDIUM: print
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LANGUAGE: English

ABSTRACT: Study Design: A histopathologic examination of a specimen that showed hypertrophy of the posterior longitudinal ligament of the cervical spine. Objectives: To illustrate the possibility of hypertrophy of the posterior longitudinal ligament as a prodromal condition to ossification of the posterior longitudinal ligament. Summary of Background Data: Despite much study, the pathology of ossification of the posterior longitudinal ligament still remains unclear. Hypertrophic change often is seen in the part of the ossified ligament; however, there have been few histopathologic reports on hypertrophy of the posterior longitudinal ligament. Some reports have suggested that hypertrophy of the posterior longitudinal ligament is a prodrome of ossification of the posterior longitudinal ligament. Methods: A 64-year-old man was admitted to the hospital because of gait disturbance and developed oliguria. In a plain radiograph, segmental ossification of the posterior longitudinal ligament was found at C4, C5, and C6. Computed tomograph myelogram revealed a soft tissue shadow, maximum 3.8 mm in diameter, on the dorsal side of the ossification of the posterior longitudinal ligament at C5 and C6. Magnetic %%%resonance%%% T1-weighted image (T1WI) showed an equivalent with the intervertebral disc on the dorsal side of ossification of the posterior longitudinal ligament. This lesion was enhanced with Gd-DTPA and confirmed as hypertrophy of the posterior longitudinal ligament. Cervical anterior decompression and %%%fusion%%% were performed using Yamura's technique. The ossified and thickened lesion was elevated and removed en bloc. Then, hematoxylin-eosin and toluidine %%%blue%%% staining was performed to detect metachromasia. Results: Macroscopic examination of the specimen revealed that soft tissue formation was connected with the C4-C5 intervertebral space and extended downward to C6-C7. Histopatholgically, collagen fibers were proliferating in the long-axis direction on both ventral and dorsal sides. This was surrounded by extended nucleus pulposus-like chondrocyte tissue, where endplate cartilage was detected around the C4 pedicle. Roux staining was low, and partial vascular and cellular infiltration was observed, although it was not marked. Conclusion: The herniated nucleus pulposus involving endplate cartilage from C4-C5 was limited to the superficial layer, and proliferation of nucleus pulposus-like chondrocytes occurred in the herniated tissue, where they might undergo a change in cell phenotype. The results of the present study support the hypothesis that hypertrophy of the posterior longitudinal ligament is a prodromal condition to ossification of the posterior longitudinal ligament.

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15935718 BIOSIS NO.: 200100107557
Imaging FRET between spectrally similar GFP molecules in single cells
AUTHOR: Harpur Ailsa G; Wouters Fred S; Bastiaens Philippe I H (Reprint)
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JOURNAL: Nature Biotechnology 19 (2): p167-169 February, 2001 2001
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ABSTRACT: Fluorescence %%%resonance%%% energy transfer (FRET) detection in %%%fusion%%% constructs consisting of green fluorescent protein (GFP) variants linked by a sequence that changes conformation upon modification by enzymes or binding of ligands has enabled detection of physiological processes such as Ca2+ ion release, and protease and kinase activity. Current FRET microscopy techniques are limited to the use of spectrally distinct GFPs such as %%%blue%%% or cyan donors in combination with green or yellow acceptors. The %%%blue%%% or cyan GFPs have the disadvantages of less brightness and of autofluorescence. Here a FRET imaging method is presented that circumvents the need for spectral separation of the GFPs by determination of the fluorescence lifetime of the combined donor/acceptor emission by fluorescence lifetime imaging microscopy (FLIM). This technique gives a sensitive, reproducible, and intrinsically calibrated FRET measurement that can be used with the spectrally similar and bright yellow and green fluorescent proteins (EYFP/EGFP), a pair previously unusable for FRET applications. We demonstrate the benefits of this approach in the analysis of single-cell signaling by monitoring caspase activity in individual cells during apoptosis.

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15920469 BIOSIS NO.: 200100092308
Dimerization with retinoid X receptors promotes nuclear localization and subnuclear targeting of vitamin D receptors
AUTHOR: Prufer Kirsten; Racz Attila; Lin Grace C; Barsony Julia (Reprint)
AUTHOR ADDRESS: NIDDK, NIH, 8 Center Dr., Bldg. 8, Rm. 422, Bethesda, MD, 20892, USA**USA
JOURNAL: Journal of Biological Chemistry 275 (52): p41114-41123 December 29, 2000 2000
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LANGUAGE: English

ABSTRACT: The vitamin D receptor (VDR) acts as heterodimer with the retinoid X receptor alpha (RXR) to control transcriptional activity of target genes. To explore the influence of heterodimerization on the subcellular distribution of these receptors in living cells, we developed

a series of fluorescent-protein %%%chimeras%%%. The steady-state distribution of the yellow fluorescent protein-RXR was more nuclear than the unliganded green fluorescent protein (GFP)-VDR. Coexpression of RXR-%%%blue%%% fluorescent protein (BFP) promoted nuclear accumulation of GFP-VDR by influencing both nuclear import and retention. Fluorescence %%%resonance%%% energy transfer microscopy (FRET) demonstrated that the unliganded GFP-VDR and RXR-BFP form heterodimers. The increase in nuclear heterodimer content correlated with an increase in basal transcriptional activity. FRET also revealed that calcitriol induces formation of multiple nuclear foci of heterodimers. Mutational analysis showed a correlation between hormone-dependent nuclear VDR foci formation and DNA binding. RXR-BFP also promoted hormone-dependent nuclear accumulation and intranuclear foci formation of a nuclear localization signal mutant receptor (nlsGFP-VDR) and rescued its transcriptional activity. Heterodimerization mutant RXR failed to alter GFP-VDR and nlsGFP-VDR distribution or activity. These experiments suggest that RXR has a profound effect on VDR distribution. This effect of RXR to promote nuclear accumulation and intranuclear targeting contributes to the regulation of VDR activity and probably the activity of other heterodimerization partners.

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15848224 BIOSIS NO.: 200100020063
Frequent polymorphism in BCR exon b2 identified in BCR-ABL positive and negative individuals using fluorescent hybridization probes
AUTHOR: Saussele S; Weisser A; Mueller M C; Emig M; La Rosee P; Paschka P; Kuhn C; Willer A; Hehlmann R; Hochhaus A (Reprint)
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JOURNAL: Leukemia (Basingstoke) 14 (11): p2006-2010 November, 2000 2000
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ABSTRACT: Recently, a polymorphic base in exon 13 of the BCR gene (exon b2 of the major breakpoint cluster region) has been identified in the eighth position before the junctional region of BCR-ABL cDNA. Cytosine replaces thymidine; the corresponding triplets are AAT (T allele) and AAC (C allele), respectively, both coding for asparagine. Therefore, this polymorphism has no implication in the primary structure of BCR and BCR-ABL proteins. However, since the alteration is located close to the %%%fusion%%% region it may have a significant influence on the annealing of PCR primers, probes for real time PCR, and antisense oligonucleotides. We have developed a RT-PCR-based screening method to easily identify polymorphic BCR and BCR-ABL alleles in CML patients and normal individuals in order to estimate their frequency. After amplification from cDNA, a melting curve of a specific fluorogenic probe mapping to the 3' end of BCR exon b2 and spanning the polymorphism readily discriminates between normal and polymorphic BCR and BCR-ABL alleles. This reporter probe is 3' labeled with %%%fluorescein%%% and placed next to 5' LC

Red640-labeled anchor probes mapping to the 5' ends of BCR exon b3 or ABL exon a2 so that %%%resonance%%% energy transfer occurs when the probes are hybridized (LightCycler technology). T and C alleles were discriminated by a melting temperature difference of the reporter probe of 3.2 K. We have investigated cDNAs derived from leukocytes from seven cell lines and a total of 229 individuals: normal donors, n = 15; BCR-ABL negative chronic myeloproliferative disorders, n = 30; BCR-ABL negative acute leukemias, n = 11; b2a2BCR-ABL positive CML, n = 93; and b3a2BCR-ABL positive CML, n = 80. The frequency of the C allele was 33.0% in BCR-ABL negative individuals, 30.6% in b2a2BCR-ABL, and 23.8% in b3a2BCR-ABL positive CML. In CML patients, 27.7% of BCR-ABL and 27.2% of BCR alleles had the C allele (NS). In total, 132 of 458 (28.8%) exons b2 of BCR or BCR-ABL alleles demonstrated this polymorphism. We conclude that a thymidine/cytosine replacement occurs frequently in BCR exon b2. Probes for real time quantitative RT-PCR should be designed not to map to the critical region in order to avoid underestimation of the number of BCR-ABL transcripts.

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15840340 BIOSIS NO.: 200100012179
Biochemistry, mutagenesis, and oligomerization of DsRed, a %%%red%%% fluorescent protein from coral
AUTHOR: Baird Geoffrey S; Zacharias David A; Tsien Roger Y (Reprint)
AUTHOR ADDRESS: University of California, San Diego, 9500 Gilman Drive, 310 Cellular and Molecular Medicine West 0647, La Jolla, CA, 92093-0647, USA **USA
JOURNAL: Proceedings of the National Academy of Sciences of the United States of America 97 (22): p11984-11989 October 24, 2000 2000
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LANGUAGE: English

ABSTRACT: DsRed is a recently cloned 28-kDa fluorescent protein responsible for the %%%red%%% coloration around the oral disk of a coral of the Discosoma genus. DsRed has attracted tremendous interest as a potential expression tracer and %%%fusion%%% partner that would be complementary to the homologous green fluorescent protein from Aequorea, but very little is known of the biochemistry of DsRed. We now show that DsRed has a much higher extinction coefficient and quantum yield than previously reported, plus excellent resistance to pH extremes and photobleaching. In addition, its 583-nm emission maximum can be further shifted to 602 nm by mutation of Lys-83 to Met. However, DsRed has major drawbacks, such as strong oligomerization and slow maturation. Analytical ultracentrifugation proves DsRed to be an obligate tetramer in vitro, and fluorescence %%%resonance%%% energy transfer measurements and yeast two-hybrid assays verify oligomerization in live cells. Also, DsRed takes days to ripen fully from green to %%%red%%% in vitro or in vivo, and mutations such as Lys-83 to Arg prevent the color change. Many potential cell biological applications of DsRed will require suppression of the tetramerization and acceleration of the maturation.

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15496983 BIOSIS NO.: 200000215296
Membrane %%%fusion%%% by proline-rich Rz1 lipoprotein, the bacteriophage lambda Rz1 gene product
AUTHOR: Bryl Krzysztof (Reprint); Kedzierska Sabina; Laskowska Magdalena; Taylor Alina
AUTHOR ADDRESS: Department of Physics and Biophysics, Warmia and Msurian University in Olsztyn, 10-957, Olsztyn, Poland**Poland
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RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The fusogenic properties of Rz1, the proline-rich lipoprotein that is the bacteriophage lambda Rz1 gene product, were studied. Light scattering was used to monitor Rz1-induced aggregation of artificial neutral (dipalmitoylphosphatidylcholine/cholesterol) and negatively charged (dipalmitoylphosphatidylcholine/cholesterol/dioleoylphosphatidylserine) liposomes. Fluorescence assays (the %%%resonance%%% energy transfer between N-(7-nitro-2,1,3-benzoxadiazol-4-yl)phosphatidylethanolamine and N-(lissamine %%%rhodamine%%% B sulfonyl)dihexadecanol-sn-glycero-3-phosphoethanolamine lipid fluorescent probes, as well as fluorescent complex formation between terbium ions and dipicolinic acid encapsulated in two liposome populations and calcein fluorescence) were used to monitor Rz1-induced lipid mixing, contents mixing and leakage of neutral and negatively charged liposomes. The results demonstrated that Rz1 caused adhesion of neutral and negatively charged liposomes with concomitant lipid mixing; membrane distortion, leading to the %%%fusion%%% of liposomes and hence their internal content mixing; and local destruction of the membrane accompanied by leakage of the liposome contents. The use of artificial membranes showed that Rz1 induced the %%%fusion%%% of membranes devoid of any proteins. This might mean that the proline stretch of Rz1 allowed interaction with membrane lipids. It is suggested that Rz1-induced liposome %%%fusion%%% was mediated primarily by the generation of local perturbation in the bilayer lipid membrane and to a lesser extent by electrostatic forces.

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15377672 BIOSIS NO.: 200000095985
Green fluorescent protein as a reporter for macromolecular localization in bacterial cells
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JOURNAL: Methods (Orlando) 20 (1): p62-72 Jan., 2000 2000

MEDIUM: print
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LANGUAGE: English

ABSTRACT: Green fluorescent protein (GFP) is a highly useful fluorescent tag for studying the localization, structure, and dynamics of macromolecules in living cells, and has quickly become a primary tool for analysis of DNA and protein localization in prokaryotes. Several properties of GFP make it an attractive and versatile reporter. It is fluorescent and soluble in a wide variety of species, can be monitored noninvasively by external illumination, and needs no external substrates. Localization of GFP %%%fusion%%% proteins can be analyzed in live bacteria, therefore eliminating potential fixation artifacts and enabling real-time monitoring of dynamics in situ. Such real-time studies have been facilitated by brighter, more soluble GFP variants. In addition, %%%red%%%-shifted GFPs that can be excited by %%%blue%%% light have lessened the problem of UV-induced toxicity and photobleaching. The self-contained domain structure of GFP reduces the chance of major perturbations to GFP fluorescence by fused proteins and, conversely, to the activities of the proteins to which it is fused. As a result, many proteins fused to GFP retain their activities. The stability of GFP also allows detection of its fluorescence in vitro during protein purification and in cells fixed for indirect immunofluorescence and other staining protocols. Finally, the different properties of GFP variants have given rise to several technological innovations in the study of cellular physiology that should prove useful for studies in live bacteria. These include fluorescence %%%resonance%%% energy transfer (FRET) for studying protein-protein interactions and specially engineered GFP constructs for direct determination of cellular ion fluxes.

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15155149 BIOSIS NO.: 199900414809
A fluorescence energy transfer study of lecithin-cholesterol vesicles in the presence of phospholipase C
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JOURNAL: Journal of Lipid Research 40 (8): p1483-1494 Aug., 1999 1999
MEDIUM: print
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LANGUAGE: English

ABSTRACT: We demonstrate Forster %%%resonance%%% energy transfer from dehydroergosterol to dansylated lecithin in lecithin-cholesterol vesicles and characterize the vesicles in the presence of the pro-nucleating enzyme, phospholipase C (PLC). Exposure to phospholipase C causes a temporary decrease in the dehydroergosterol to dansyl fluorescence ratio followed by an increase to and above the initial value. The temporary

decrease in the fluorescence ratio results from an increase in the dansylated lecithin intensity that coincides with a dansyl %%%blue%%% shift. The extent of the %%%blue%%% shift correlates with the level of diacylglycerol generated in situ by PLC, suggesting an increased association between dansylated lecithin and cholesterol as membrane fluidity increases and membrane polarity decreases. The subsequent increase in the fluorescence ratio results from both an increase in the dehydroergsterol intensity and a concomitant decrease in the dansylated lecithin intensity of equal magnitude. This signifiesa reduction in energy transfer from dehydroergosterol to dansylated lecithin and indicates an increased separation between the two fluorophores. The increase in the fluorescence ratio persists beyond the time scales for vesicle aggregation and %%%fusion%%%, as measured by turbidity, and precedes the onset of macroscopic cholesterol crystals observed with an optical microscope. Thus, the increased separation between dehydroergosterol and dansylated lecithin is consistent with a mechanism of cholesterol nucleation from the vesicles. Moreover, the onset and rate of increase in the fluorescence ratio correlate with the cholesterol:lecithin mole ratio of the vesicles. Fluorescence energy transfer from dehydroergosterol to dansylated lecithin therefore shows potential as a methodology for measuring cholesterol nucleation in model bile.

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15154858 BIOSIS NO.: 199900414518
On the maximum size of proteins to stay and fold in the cavity of GroEL underneath GroES
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JOURNAL: Journal of Biological Chemistry 274 (30): p21251-21256 July 23, 1999 1999
MEDIUM: print
ISSN: 0021-9258
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RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: GroEL encapsulates non-native protein in a folding cage underneath GroES (cis-cavity). Here we report the maximum size of the non-native protein to stay and fold in the cis-cavity. Using total soluble proteins of Escherichia coli in denatured state as binding substrates and protease resistance as the measure of polypeptide held in the cis-cavity, it was estimated that the cis-cavity can accommodate up to apprx57-kDa non-native proteins. To know if a protein with nearly the maximum size can complete folding in the cis-cavity, we made a 54-kDa protein in which green fluorescent protein (GFP) and its %%%blue%%% fluorescent variant were fused tandem. This %%%fusion%%% protein was captured in the cis-cavity, and folding occurred there. Fluorescence %%%resonance%%% energy transfer proved that both GFP and %%%blue%%% fluorescent protein moieties of the same fused protein were able to fold into native structures in the cis-cavity. Consistently, simulated packing

of crystal structures shows that two native GFPsjust fit in the cis-cavity. A %%%fusion%%% protein of three GFPs (82 kDa) was also attempted, but, as expected, it was not captured in the cis-cavity.

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15136206 BIOSIS NO.: 199900395866
Vesicular-integral membrane protein, VIP36, recognizes high-mannose type glycans containing alpha1fwdarw2 mannosyl residues in MDCK cells
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JOURNAL: Glycobiology 9 (8): p833-839 Aug., 1999 1999
MEDIUM: print
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LANGUAGE: English

ABSTRACT: The 36 kDa vesicular-integral membrane protein, VIP36, has been originally isolated from MDCK cells as a component of glycolipid-enriched detergent-insoluble complexes containing apical marker proteins, and its luminal domain shows homology to leguminous plant lectins and ERGIC-53. As the first step to identify the functional role of VIP36, the carbohydrate binding specificity of VIP36 was investigated using a %%%fusion%%% protein of glutathione-S-transferase and luminal domain of VIP36 (Vip36). It was found that VIP36 recognizes high-mannose type glycans containing alpha1fwdarw2 Man residues and alpha-amino substituted asparagine. The binding of Vip36 to high-mannose type glycans was independent of Ca2+ and the optimal condition was pH 6.0 at 37degreeC. The concentration at which half inhibition of the binding by Man7-9cntdotGlcNAc2cntdotNAccntdotAsn occurred was 1.0 X 10-9 M. The association constant between Man7-9cntdotGlcNAc2 in porcine thyroglobulin and immobilized Vip36 was 2.1 X 108 M-1 as determined by means of a biosensor based on surface plasmon %%%resonance%%%. These results indicate that VIP36 functions as an intracellular lectin recognizing glycoproteins which possess high-mannose type glycans, (Manalpha1fwdarw2)2-4cntdotMan5cntdotGlcNAc2.

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14939562 BIOSIS NO.: 199900199222
Frequency encoding for simultaneous display of multimodality images
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JOURNAL: Journal of Nuclear Medicine 40 (3): p442-447 March, 1999 1999
MEDIUM: print

ISSN: 0161-5505
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: An original method for simultaneous display of functional and anatomic images, based on frequency encoding (FE), merges color PET with T1-weighted MR brain images, and grayscale PET with multispectral color MR images. A comparison with two other methods reported in the literature for image %%%fusion%%% (averaging and intensity modulation techniques) was performed. Methods: For FE, the Fourier transform of the merged image was obtained summing the low frequencies of the PET image and the high frequencies of the MR image. For image averaging, the merged image was obtained as a weighted average of the intensities of the two images to be merged. For intensity modulation, the %%%red%%%, green and %%%blue%%% components of the color image were multiplied on a pixel-by-pixel basis by the grayscale image. A comparison of the performances of the three techniques was made by three independent observers assessing the conspicuity of specific MRI and PET information in the merged images. For evaluation purposes, images from seven patients and a computer-simulated MRI/PET phantom were used. Data were compared with a chi-square test applied to ranks. Results: For the depiction of MRI and PET information when merging color PET and T1-weighted MR images, FE was rated superior to intensity modulation and averaging techniques in a significant number of comparisons. For merging grayscale PET with multispectral color MR images, FE and intensity modulation were rated superior to image averaging in terms of both MRI and PET information. Conclusion: The data suggest that improved simultaneous evaluation of MRI and PET information can be achieved with a method based on FE.

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14893502 BIOSIS NO.: 199900153162
Relocating the active site of activated protein C eliminates the need for its protein S cofactor: A fluorescence %%%resonance%%% energy transfer study
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JOURNAL: Journal of Biological Chemistry 274 (9): p5462-5468 Feb. 26, 1999
1999
MEDIUM: print
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The effect of replacing the gamma-carboxyglutamic acid domain of activated protein C (APC) with that of prothrombin on the topography of the membrane-bound enzyme was examined using fluorescence %%%resonance%%% energy transfer. The average distance of closest approach (assuming kappa2 = 2/3) between a %%%fluorescein%%% in the active site of the %%%chimera%%% and octadecylrhodamine at the membrane surface was 89 ANG

compared with 94 A for wild-type APC. The gamma-carboxyglutamic acid domain substitution therefore lowered and/or reoriented the active site, repositioning it close to the 84 ANG observed for the APC-protein S complex. Protein S enhances wild-type APC cleavage of factor Va at Arg306, but the inactivation rate of factor Va Leiden by the %%%chimera%%% alone is essentially equal to that by wild-type APC plus protein S. These data suggest that the activities of the %%%chimera%%% and of the APC protein S complex are equivalent because the active site of the %%%chimeric%%% protein is already positioned near the optimal location above the membrane surface to cleave Arg306. Thus, one mechanism by which protein S regulates APC activity is by relocating its active site to the proper position above the membrane surface to optimize factor Va cleavage.

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14883328 BIOSIS NO.: 199900142988
Liposomes fuse with sperm cells and induce activation by delivery of impermeant agents
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JOURNAL: Biochimica et Biophysica Acta 1417 (1): p77-88 Feb. 4, 1999 1999
MEDIUM: print
ISSN: 0006-3002
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Sperm cell activation is a critical step in fertilization. To directly investigate the cell signaling events leading to sperm activation it is necessary to deliver membrane impermeant agents into the cytoplasm. In this study, the use of liposomes as possible agent-loading vectors was examined using (1) the octadecylrhodamine B (R18) and NBD phosphatidylethanolamine (NBD DHPE)/%%%rhodamine%%% phosphatidylethanolamine (rhod DHPE) %%%fusion%%% assays in bulk samples, (2) membrane transfer of fluorescence from liposome membranes labeled with R18 and %%%rhodamine%%%-tagged phosphatidylethanolamine (TRITC DHPE), and (3) lumenal transfer of impermeant calcium ions from liposomes to sperm cells, a process that stimulated sperm cell activation. Intermediate-sized unilamellar liposomes (98.17 +- 15.34 nm) were prepared by the detergent-removal technique using sodium cholate as the detergent and a phosphatidylcholine/phosphatidylethanolamine/cholesterol (2:1:1 mole ratio) lipid composition. In the R18 %%%fusion%%% assays, self-quenching increased logarithmically with increasing concentrations of R18 in the liposome membranes; addition of unlabeled sperm to R18-labeled liposomes lead to a rapid release of self-quenching. In the NBD DHPE/rhod DHPE %%%resonance%%% energy transfer (RET) %%%fusion%%% assay, RET was rapidly reduced under similar conditions, In addition, individual sperm became fluorescent when TRITC DHPE-labeled liposomes were incubated with unlabeled sperm cells. Incubation of sperm cells with empty liposomes did not significantly affect sperm cell activation and did not alter cell morphology. However, incubation with Ca (10 mM)-loaded

liposomes resulted in a time-dependent increase in sperm cell activation (7.5-fold over controls after 15 min). We conclude that liposomes can be used for direct loading of membrane-impermeant agents into sea squirt sperm cell cytoplasm, and that delivery occurs via %%%fusion%%% and content intermixing.

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14748288 BIOSIS NO.: 199900007948
Swing of the lever arm of a myosin motor at the isomerization and phosphate-release steps
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JOURNAL: Nature (London) 396 (6709): p380-383 Nov. 26, 1998 1998
MEDIUM: print
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LANGUAGE: English

ABSTRACT: In muscle, the myosin head ('crossbridge') performs the 'working stroke', in which ATP is hydrolysed to generate the sliding of actin and myosin filaments. The myosin head consists of a globular motor domain and a long lever-arm domain. The 'lever-arm hypothesis' predicts that during the working stroke, the lever-arm domain tilt against the motor domain, which is bound to actin in a fixed orientation. To detect this working stroke in operation, we constructed %%%fusion%%% proteins by connecting Aequorea victoria green fluorescent protein and %%%blue%%% fluorescent protein to the amino and carboxyl termini of the motor domain of myosin II of Dictyostelium discoideum, a soil amoeba, and measured the fluorescence %%%resonance%%% energy transfer between the two fluorescent proteins. We show here that the carboxy-terminal fluorophore swings at the isomerization step of the ATP hydrolysis cycle, and then swings back at the subsequent step in which inorganic phosphate is released, thereby mimicking the swing of the lever arm. The swing at the phosphate-release step may correspond to the working stroke, and the swing at the isomerization step to the recovery stroke.

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14697410 BIOSIS NO.: 199800491657
Magnetic %%%resonance%%% imaging and biological changes in injured intervertebral discs under normal and increased mechanical demands
AUTHOR: Olsewski John M; Schendel Michael J (Reprint); Wallace Larry J; Ogilvie James W; Gundry Cooper R
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JOURNAL: Spine 21 (17): p1945-1951 Sept. 1, 1996 1996
MEDIUM: print

ISSN: 0362-2436
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RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Study Design. An animal model was used to examine the short-term tissue response to changes in the mechanical environment after the structure (disc) is mechanically injured. Objectives. To observe changes in an injured intervertebral disc and the corresponding motion segment when the mechanical demands of the disc were increased by %%%fusion%%% of the adjacent motion segments. Summary of Background Data. Disc degeneration has been modeled in animals by producing a tear in the anulus via laminectomy, laparotomy, or posterolaterally. Methods of altering and quantifying the mechanics of the intervertebral joint by use of internal fixation and %%%fusion%%% in the %%%canine%%% have been developed. Methods. Eight dogs divided into two groups (a study and a control group) had anular stab wounds (L2-L3). The study group was surgically instrumented posteriorly from L3 to L7. Magnetic %%%resonance%%% imaging studies were conducted for all animals before and periodically after the surgical procedures. At the end of the study, the segments were processed histologically and biochemically. Results. Anular bulging was seen on magnetic %%%resonance%%% imaging in all control animals 4 months after injury and did not progress out until 6 months after-injury. Similar changes were seen in study animals, but 75% were herniated by 6 months. Histologic changes correlated with magnetic %%%resonance%%% imaging changes. No significant difference in water or proteoglycan content of the disc tissue between groups was found. Conclusions. Progression from the bulging of the anulus to herniation was not evident in damaged discs not subjected to adjacent fusions. No change in water or proteoglycan content as a function of altered mechanical state was found, suggesting the short-term effect of the altered mechanics is on the mechanical structure and not on the cells or extracellular matrix.

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14648715 BIOSIS NO.: 199800442962
Visualization of Pit-1 transcription factor interactions in the living cell nucleus by fluorescence %%%resonance%%% energy transfer microscopy
AUTHOR: Day Richard N (Reprint)
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JOURNAL: Molecular Endocrinology 12 (9): p1410-1419 Sept., 1998 1998
MEDIUM: print
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LANGUAGE: English

ABSTRACT: The pituitary-specific transcription factor Pit-1 forms dimers when interacting with specific DNA elements and has been shown to associate with several other nuclear proteins. Recently, techniques have become available that allow visualization of protein-protein interactions as they occur in single living cells. In this study, the technique of

fluorescence %%%resonance%%% energy transfer (FRET) microscopy was used to visualize the physical interactions of Pit-1 proteins fused to spectral variants of the jellyfish green fluorescent protein (GFP) that emit green or %%%blue%%% light (%%%blue%%% fluorescent protein (BFP)). An optimized imaging system was used to discriminate fluorescence signals from single cells coexpressing the BFP- and GFP-%%%fusion%%% proteins, and the contribution of spectral overlap to background fluorescence detected in the FRET images was established. Energy transfer signals from living cells expressing a %%%fusion%%% protein in which GFP was tethered to BFP by short protein linker was used to demonstrate acquisition of FRET signals. Genetic vectors encoding GFP- and BFP-Pit-1 proteins were prepared, and biological function of the %%%fusion%%% proteins was confirmed. FRET microscopy of HeLa cells coexpressing the GFP- and BFP-Pit-1 demonstrated energy transfer, which required the two fluorophores to be separated by less than 100 ANG. Biochemical studies previously demonstrated that Pit-1 physically interacts with both c-Ets-1 and the estrogen receptor. FRET imaging of cells coexpressing BFP-Pit-1 and GFP-Ets-1 demonstrated energy transfer between these %%%fusion%%% proteins, a result consistent with their association in the nucleus of these living cells. In contrast, there was no evidence for energy transfer between the BFP-Pit-1 and an estrogen receptor-GFP %%%fusion%%% proteins. It is likely that the FRET imaging approach described here can be applied to many different protein-partner pairs in a variety of cellular contexts.

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14547215 BIOSIS NO.: 199800341462
Multifunctional g3p-peptide tag for current phage display systems
AUTHOR: Beckmann Christiane; Haase Bernd; Timmis Kenneth N; Tesar Michael (Reprint)
AUTHOR ADDRESS: Div. Microbiol., GBF-Natl. Res. Centre Biotechnol., Mascheroder Weg 1, D-38124 Braunschweig, Germany**Germany
JOURNAL: Journal of Immunological Methods 212 (2): p131-138 March 15, 1998 1998
MEDIUM: print
ISSN: 0022-1759
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: We have previously described a monoclonal antibody (mAb), 10C3, directed against the gene-3 protein (g3p) of filamentous phage M13, which was produced to study g3p %%%fusion%%% protein expression in Escherichia coli and its incorporation in the phage capsid (Tesar, M., Beckmann, C., Rottgen, P., Haase, B., Faude, U., Timmis, K., 1995. Monoclonal antibody against pIII of filamentous phage: an immunological tool to study pIII %%%fusion%%% protein expression in phage display systems. Immunology 1, 53-54). In this study we report mapping of the antigenic epitope of the mAb 10C3, by means of short overlapping peptide-sequences (Frank, R., Overwin, H., 1996. Spot synthesis. In: Morris, G.E. (Ed.), Methods in Molecular Biology, Vol. 66: Epitope Mapping Protocols. Humana Press, Totowa, NJ, pp. 149-169.) comprising the C-terminal half of the g3-protein. A minimal recognizable peptide was found which is represented

in the 11 amino acid sequence from positions 292 to 302 of g3p (Wezenbeek van, P.M.G.P., Hulsebos, T.J.M., Schoenmakers, J.G.G., 1980. Nucleotide sequence of the filamentous bacteriophage Ml 3 DNA genome: comparison with phage fd. Gene II, 129-148). In order to use the antibody also for detection and purification of recombinant proteins, such as single chain antibodies, the epitope was introduced as a tag sequence into the phagemid pHEN) (Hoogenboom, H.R., Griffith, A.D., Johnson, K., Chiswell, D.J., Hudson, P., Winter, G., 1991. Multi-subunit proteins on the surface of the filamentous phage: methodologies for displaying antibody (Fab) heavy and light chains. Nucleic Acid Res. 19, 4133-4137; Nissim, A., Hoogenboom, H.R., Tomlinson, I.M., Flynn, G., Midgley, C., Lane, D., Winter. G., 1994. Antibody fragments from a single pot phage display library as immunochemical reagents. EMBO J. 13 (3) 692-698). Purified single chain antibodies containing this tag were detectable down to a concentration of 2 mg ml-1 under non-denaturing conditions (ELISA) or 4 ng per lane on immunoblots. The high sensitivity of the antibody for the peptide tag was reflected in the antibody affinity constant KD of 6.80 X 10-10 M, which was determined by real time biomolecular interaction analysis (BIA) based on surface plasmon %%%resonance%%% (SPR) (Karlsson, R., Falt, A., 1997. Experimental design for kinetic analysis of protein-protein interactions with surface plasmon %%%resonance%%% biosensors. J. Immunol. Methods 200, 121-133). Finally, recombinant proteins in E. coli periplasmic extracts could be purified in a single step-by affinity purification using immobilized mAb 10C3. These studies demonstrated that the new peptide-tag and its corresponding mAb represents a versatile tool for the detection of recombinant proteins selected by phage display technology.

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14508279 BIOSIS NO.: 199800302526
Bcl-2 and Bax interactions in mitochondrial probed with green fluorescent protein and fluorescence %%%resonance%%% energy transfer
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AUTHOR ADDRESS: Dep. Cell Biol. Anatomy, Lineberger Comprehensive Cancer Cent., Univ. North Carolina, Chapel Hill, NC 27599, USA**USA
JOURNAL: Nature Biotechnology 16 (6): p547-552 June, 1998 1998
MEDIUM: print
ISSN: 1087-0156
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RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: It has been hypothesized that interaction of Bcl-2 and Bax may regulate apoptosis. The spatial and temporal interaction of Bcl-2 and Bax at the single cell level has not, however, been demonstrated. To achieve this goal, we have developed two-%%%fusion%%% FRET (fluorescence %%%resonance%%% energy transfer). Using green fluorescent protein (GFP)-Bax and %%%blue%%% fluorescent protein (BFP)-Bcl-2 %%%fusion%%% proteins coexpressed in the same cell, we demonstrate a direct interaction between Bcl-2 and Bax in individual mitochondria. Mitochondrially localized cytochrome c-GFP and BFP-Bcl-2 showed little or no FRET, while nuclear-localized GFP-human papillomavirus E6 and

BFP-Bcl-2 did not interact when coexpressed in the same cell. These findings indicate that two-%%%fusion%%% FRET provides an opportunity to examine the interaction between two different proteins coexpressed in single intact mammalian cells.

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14427961 BIOSIS NO.: 199800222208
%%%Fusion%%% of phospholipid vesicles induced by the ribosome inactivating protein saporin
AUTHOR: Liu Guohong; Hao Qiang; Zhang Yan; Gao Gui; Yan Ganglin; Yao Qizhi; Li Qingshan (Reprint)
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JOURNAL: Biochemistry and Molecular Biology International 42 (5): p873-880 Aug., 1997 1997
MEDIUM: print
ISSN: 1039-9712
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The single chain ribosome-inactivating protein Saporin-S6 (SO-6) induces the %%%fusion%%% of acid phospholipid vesicles. The extent of %%%fusion%%% was measured by %%%resonance%%% energy transfer assay between the N-(7-nitro-2-1,3-benzoxadiazol-4-yl)-dimyristoylphosphatidyl lithanolamine (NBDPE)(donor) and N-(lissamine %%%rhodamine%%% B sulphonyl)-diacylphoshaidylethanolamine (Rh-PE) (acceptor) incorporated in the vesicle. The saturated lipid/protein molar ratio is approx. 100:1. The time course of %%%fusion%%% of vesicles induced by the protein showed that the process was completed within 10 minutes, and the size of the particles in the medium was enlarged which conforms the occurrence of the %%%fusion%%% occurring. The %%%fusion%%% is temperature dependent and the liquid-crystalline state lipid is more apt to fuse than the gel phase lipid. The effect of SO-6 is also dependent on ionic strength and pH, high salt concentration and basic pH may abolish %%%fusion%%%, which suggests that both electrostatic and hydrophobic components may be involved in the process.

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14112460 BIOSIS NO.: 199799746520
The amino-terminal %%%fusion%%% domain peptide of human immunodeficiency virus type 1 gp41 inserts into the sodium dodecyl sulfate micelle primarily as a helix with a conserved glycine at the micelle-water interface
AUTHOR: Chang Ding-Kwo (Reprint); Chen Shu-Fang; Chien Wei-Jyun
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JOURNAL: Journal of Virology 71 (9): p6593-6602 1997 1997
ISSN: 0022-538X
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RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: A peptide based on the N-terminal %%%fusion%%% domain of gp41 of human immunodeficiency virus type 1 (HIV-1) and its tryptophan analog were synthesized to examine the secondary structure in the micellar environment. Nuclear magnetic %%%resonance%%% (NMR), circular dichroism and electron paramagnetic %%%resonance%%% experiments indicated that the gp41 %%%fusion%%% peptide inserted into the micelle primarily as a helix (59%), with substantial beta-structure (26.7%). Deep penetration of the peptide into the apolar hydrocarbon core was supported by the results of fluorescence experiments in which the tryptophan analog exhibited a %%%blue%%% shift of about 30 nm in the presence of a sodium dodecyl sulfate micelle, in 1,2-dimyristoyl-rac-glycero-3-phosphocholine, and in 1,2-dipalmitoyl-sn-glycero-3-phospho-L-serine vesicular solutions. The results of spin label-attenuated 1H %%%resonance%%% experiments show that the region C-terminal to G16, which contains a turn structure, exhibited substantial interaction with the micelle, suggesting that it lies on the surface of micelle. Molecular simulation based on data from NMR experiments revealed a flexible hinge at residues 15 and 16 (alanine and glycine, respectively) from the N terminus of the peptide located at the micelle-solution interface. The highly conserved A15-GI6 dipeptide may play a role in the function of %%%fusion%%% domain of HIV-1 envelope glycoprotein.

13/7/85
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13926482 BIOSIS NO.: 199799560542
Cytoplasmic delivery of calcein mediated by liposomes modified with a pH-sensitive poly(ethylene glycol) derivative
AUTHOR: Kono Kenji (Reprint); Igawa Toshihiro; Takagishi Toru
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JOURNAL: Biochimica et Biophysica Acta 1325 (2): p143-154 1997 1997
ISSN: 0006-3002
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Previously, as a new type of pH-sensitive liposome, we prepared egg yolk phosphatidylcholine (EYPC) liposomes bearing succinylated poly(glycidol), that is a poly(ethylene glycol) derivative having carboxyl groups, and showed that %%%fusion%%% ability of the liposomes increases under weakly acidic and acidic conditions (Kono, K., Zenitani, K. and Takagishi, T. (1994) Biochim. Biophys. Acta 1193, 1-9). In this study, we examined intracellular delivery of a water-soluble molecule, calcein, mediated by the succinylated poly(glycidol)-modified liposomes. When CV- I cells, an established line of African green monkey kidney cells, were incubated with bare EYPC liposomes containing calcein at 37 degree C, only weak and vesicular fluorescence of calcein was observed by using a fluorescence microscope. In contrast, the cells treated with the polymer-modified liposomes containing calcein displayed more intensive and diffuse fluorescence, indicating that calcein was transferred into the cytoplasm. Uptake of the polymer-modified liposomes by the cells was

shown to decrease slightly as amount of the polymer fixed on the liposome increases. However, the fluorescence of calcein observed in the liposome-treated cell was, on the contrary, enhanced as amount of the polymer fixed on the liposome increases, indicating that the liposome modified with a higher amount of the polymer transfers its content into cytoplasm more efficiently after internalization into the cell. %%%Fusion%%% assay by %%%resonance%%% energy transfer using N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)phosphatidylethanolamine and lissamine %%%rhodamine%%% B-sulfonylphosphatidylethanolamine suggested occurrence of %%%fusion%%% between the polymer-modified liposomes and endosomal and/or lysosomal membranes. Moreover, the liposome with a higher polymer content revealed higher percent %%%fusion%%% after internalization into the cell. These results imply that the polymer-modified liposomes transfer the content into the cytoplasm by fusing with the endosomal membrane after internalization into the cells through an endocytic pathway.

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13468664 BIOSIS NO.: 199699102724
Fluorescence %%%resonance%%% energy transfer between %%%blue%%%-emitting and %%%red%%%-shifted excitation derivatives of the green fluorescent protein
AUTHOR: Mitra Robi D; Silva Christopher M; Youvan Douglas C (Reprint)
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JOURNAL: Gene (Amsterdam) 173 (1): p13-17 1996 1996
ISSN: 0378-1119
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: We report fluorescent %%%resonance%%% energy transfer (FRET) between two linked variants of the green fluorescent protein (GFP). The C terminus of a %%%red%%%-shifted variant of GFP (RSGFP4) is fused to a flexible polypeptide linker containing a Factor X-a protease cleavage site. The C terminus of this linker is in turn fused to the N terminus of a %%%blue%%% variant of GFP (BFP5). The gene product has spectral properties that suggest energy transfer is occurring from BFP5 to RSGFP4. Upon incubation with Factor X-a, the protein is cleaved, and the two fluorescent proteins dissociate. This is accompanied by a marked decrease in energy transfer. The RSGFP4::BFP5 %%%fusion%%% protein demonstrates the feasibility of using FRET between two GFP derivatives as a tool to monitor protein-protein interactions; in addition, this construct may find applications as an intracellular screen for protease inhibitors.

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13463873 BIOSIS NO.: 199699097933
Rapid identification of phosphopeptide ligands for SH2 domains: Screening of peptide libraries by fluorescence-activated bead sorting

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JOURNAL: Journal of Biological Chemistry 271 (28): p16500-16505 1996 1996
ISSN: 0021-9258
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LANGUAGE: English

ABSTRACT: A method for the identification of high-affinity ligands to SH2 domains by fluorescence-activated bead sorting (FABS) was established. Recombinant SH2 domains, expressed as glutathione S-transferase (GST) %%%fusion%%% proteins, were incubated with a phosphotyrosine (Y*)-containing peptide library. 6.4 times 10-5 individual peptides of nine amino acids in length (EPX-6Y*X-19X-7X-19X-7X-6) were each displayed on beads. Phosphopeptide interaction of a given SH2 domain was monitored by binding of %%%fluorescein%%% isothiocyanate-labeled antibodies directed against GST. High-fluorescence beads were isolated by flow cytometric sorting. Subsequent pool sequencing of the selected beads revealed a distinct pattern of phosphotyrosine-containing motifs for each individual SH2 domain: the SH2 domain of the adapter protein Grb2 predominantly selected beads with the sequence Y*ENDP, whereas the C-terminal SH2 domain of the tyrosine kinase Syk selected Y*EELD, each motif representing the most frequently found residues C-terminal to the phosphotyrosine. For deconvolution studies, soluble phosphopeptides comprising variations of the Grb2 motifs were resynthesized and analyzed by surface plasmon %%%resonance%%%.

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13352986 BIOSIS NO.: 199698820819
Non-phospholipid fusogenic liposomes
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JOURNAL: Biochimica et Biophysica Acta 1280 (1): p107-114 1996 1996
ISSN: 0006-3002
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: We have demonstrated the capacity of non-phospholipid liposomes composed primarily of dioxyethylene acyl ethers and cholesterol to fuse with membranes composed primarily of phospholipid. Phase-contrast microscopy, freeze-fracture electron microscopy and a macromolecular probe indicate that these non-phospholipid liposomes can fuse with the plasma membranes of erythrocytes and fibroblasts. Furthermore, fluorescence probe experiments have demonstrated %%%fusion%%% between phosphatidylcholine liposomes and nonphospholipid liposomes. Mixing of internal contents was shown by a terbium/dipicolinate assay. Mixing of

membrane lipid components was demonstrated by measuring (i) fluorescence %%%resonance%%% energy transfer between N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)phosphatidyl-ethanolamine and N-(lissamine %%%rhodamine%%% B sulfonyl)phosphatidylethanolamine, after phosphatidylcholine liposomes were mixed with non-phospholipid liposomes, and (ii) reduced concentration quenching of rhodaminephosphatidylethanolamine and octadecylrhodamine incorporated into phosphatidylcholine liposomes after mixing with the non-phospholipid liposomes. The degree of apparent %%%fusion%%% reported by the different probe techniques ranged from 25% to 64%.

13/7/89
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13303393 BIOSIS NO.: 199698771226
%%%Resonance%%% energy transfer imaging of phospholipid vesicle interaction with a planar phospholipid membrane: Undulations and attachment sites in the region of calcium-mediated membrane-membrane adhesion
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JOURNAL: Journal of General Physiology 107 (3): p329-351 1996 1996
ISSN: 0022-1295
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Membrane %%%fusion%%% of a phospholipid vesicle with a planar lipid bilayer is preceded by an initial prefusion stage in which a region of the vesicle membrane adheres to the planar membrane. A %%%resonance%%% energy transfer (RET) imaging microscope, with measured spectral transfer functions and a pair of radiometrically calibrated video cameras, was used to determine both the area of the contact region and the distances between the membranes within this zone. Large vesicles (5-20 mu-m diam) were labeled with the donor fluorophore coumarin-phosphatidylethanolamine (PE), while the planar membrane was labeled with the acceptor %%%rhodamine%%%-PE. The donor was excited with 390 nm light, and separate images of donor and acceptor emission were formed by the microscope. Distances between the membranes at each location in the image were determined from the RET rate constant (k-t) computed from the acceptor:donor emission intensity ratio. In the absence of an osmotic gradient, the vesicles stably adhered to the planar membrane, and the dyes did not migrate between membranes. The region of contact was detected as an area of planar membrane, coincident with the vesicle image, over which %%%rhodamine%%% fluorescence was sensitized by RET. The total area of the contact region depended biphasically on the Ca-2+ concentration, but the distance between the bilayers in this zone decreased with increasing (Ca-2+). The changes in area and separation were probably related to divalent cation effects on electrostatic screening and binding to charged membranes. At each (Ca-2+), the intermembrane separation varied between 1 and 6 nm within each contact region, indicating membrane undulation prior to adhesion. Intermembrane separation distances ltoreq 2 nm were localized to discrete sites that formed in an ordered arrangement throughout the contact region. The area of the contact region occupied by these punctate attachment sites was

increased at high (Ca-2+). Membrane %%%fusion%%% may be initiated at these sites of closest membrane apposition.

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13254448 BIOSIS NO.: 199698722281
Engineering green fluorescent protein for improved brightness, longer wavelengths and fluorescence %%%resonance%%% energy transfer
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JOURNAL: Current Biology 6 (2): p178-182 1996 1996
ISSN: 0960-9822
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Background: Variants of the green fluorescent protein (GFP) with different colors would be very useful for simultaneous comparisons of multiple protein fates, developmental lineages and gene expression levels. The simplest way to shift the emission color of GFP is to substitute histidine or tryptophan for the tyrosine in the chromophore, but such %%%blue%%%-shifted point mutants are only dimly fluorescent. The longest wavelengths previously reported for the excitation and emission peaks of GFP mutants are 488 and 511 nm, respectively. Results: Additional substitutions, mainly in residues 145-163, have improved the brightness of the %%%blue%%%-shifted GFP mutants with histidine and tryptophan in place of tyrosine 66. Separate mutations have pushed the excitation and emission peaks of the most %%%red%%%-shifted mutant to 504 and 514 nm, respectively. At least three different colors of GFP mutants can now be cleanly distinguished from each other under the microscope, using appropriate filter sets. A %%%fusion%%% protein consisting of linked %%%blue%%%- and green-fluorescent proteins exhibits fluorescence %%%resonance%%% energy transfer, which is disrupted by proteolytic cleavage of the linker between the two domains. Conclusions: Our results demonstrate that the production of more and better GFP variants is possible and worthwhile. The production of such variants facilitates multicolor imaging of differential gene expression, protein localization or cell fate. Fusions between mutants of different colors may be useful substrates for the continuous in situ assay of proteases. Demonstration of energy transfer between GFP variants is an important step towards a general method for monitoring the mutual association of %%%fusion%%% proteins.

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12762130 BIOSIS NO.: 199598229963
A new approach to the quantitation of membrane based on fluorescence energy transfer measurements
AUTHOR: Razinkov V I; Molotkovskii Yu G
AUTHOR ADDRESS: M.M. Shemyakin and Yu. A. Ovchinnikov Inst. Bioorg. Chem.,

Russ. Acad. Sci., Moscow, Russia**Russia
JOURNAL: Biologicheskie Membrany (Moscow) 11 (5): p538-547 1994 1994
ISSN: 0233-4755
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: Russian

ABSTRACT: A new modification of the method for quantitation of membrane %%%fusion%%% based on measuring the %%%resonance%%% energy transfer between two fluorescent probes incorporated In the same particle population was developed. To improve accuracy of the method, the sizes of fusing particles, as determined with laser spectroscopy, were taken into account. The method was applied to the Ca-2+-induced %%%fusion%%% of two populations of phosphatidylserine vesicles, one of which was doped with two different %%%Rhodamine%%% lipid probes comprising a donor-acceptor pair.

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12436969 BIOSIS NO.: 199497458254
In vivo evaluation of anterior cervical fusions with hydroxylapatite graft material
AUTHOR: Cook Stephen D (Reprint); Dalton Jeanette E; Tan Edward H; Tejeiro William V; Young Michael J; Whitecloud Thomas S III
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JOURNAL: Spine 19 (16): p1856-1866 1994 1994
ISSN: 0362-2436
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Study Design: The efficacy of solid hydroxylapatite (HA) blocks for obtaining cervical interbody %%%fusion%%% was studied in a %%%canine%%% model. Each of 21 adult colony reared hounds received a hydroxylapatite implant and an autogenous bone graft in the C3-C4 and C5-C6 disc interspaces. Seven dogs each were killed at 6, 12, and 26 weeks postoperation. Objective: This study determined the rate, mechanical strength, and histologic characteristics of cervical interbody fusions achieved using a hydroxylapatite (HA) block and compared the results to those obtained with autogenous iliac crest bone graft. Summary of Background Data: The use of corticocancellous autograft has been successful in a high percentage of anterior cervical interbody fusions. Calcium phosphate ceramics may provide an alternative to autogenous and allogenous tissue. These materials are biocompatible and capable of direct intimate bonding with bone because of their chemical similarity to bone mineral. Methods: Radiographic evaluation including plain radiographs, computed tomography (CT), and magnetic %%%resonance%%% imaging (MRI) studies were used to determine %%%fusion%%% quality. Specimens were also subjected to mechanical testing to determine bending and torsional stiffness as well as ultimate load to failure. Histologic evaluation included integrity and incorporation of the graft materials and interface characterization. Results: The CT images were well correlated with plain radiographs and demonstrated progressive

incorporation of the graft materials with time. Magnetic %%%resonance%%% images were of little value in evaluating quality of %%%fusion%%%; the autograft sites demonstrated progressive disc height loss with time. Minimal disc height loss was observed with HA blocks. There was no statistical difference in torsional stiffness for the HA blocks and autogenous bone grafts at any time period. In bending, the HA block sites were significantly stiffer at 6 weeks (P lt 0.005). There was no statistical difference in the ultimate failure load for the HA and autogenous bone grafts. Histologically, the HA blocks demonstrated areas of direct bone apposition with increased bone appositions and implant incorporation with time. At 6 and 12 weeks postoperation, the autograft sites demonstrated areas of graft resorption and some new bone formations. By 26 weeks, new bone was continuous with the vertebral endplates. Conclusions: The results indicate that HA blocks may provide an alternative to autogenous graft materials for anterior cervical interbody %%%fusion%%%. Block slippage and/or fracture may occur in a small number of patients but is primarily related to insertion technique and does not appear to significantly alter the final result.

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12406679 BIOSIS NO.: 199497427964
Electron spin %%%resonance%%% methods for studying virus-cell membrane %%%fusion%%%
BOOK TITLE: Methods in Enzymology; Membrane %%%fusion%%% techniques, Part A
AUTHOR: Ohnishi Shun-Ichi (Reprint); Kuroda Kazumichi
BOOK AUTHOR/EDITOR: Duzgunes N (Editor)
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SERIES TITLE: Methods in Enzymology 220 p331-342 1993
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Academic Press Ltd., 14 Belgrave Square, 24-28 Oval Road, London NW1 70X, England, UK
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12168175 BIOSIS NO.: 199497189460
Fusogenic virosomes prepared by partitioning of vesicular stomatitis virus G protein into preformed vesicles
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JOURNAL: Journal of Biological Chemistry 269 (6): p4050-4056 1994 1994
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Virosomes were prepared by the insertion of vesicular stomatitis virus glycoprotein, a pH-sensitive %%%fusion%%% protein, into preformed liposomes. The fusogenic activity of these virosomes was characterized in cell-free %%%fusion%%% assays using liposomal targets. %%%Fusion%%% was monitored by concentration-dependent changes in the efficiency of %%%resonance%%% energy transfer between N-(lissamine %%%rhodamine%%% B sulfonyl)-phosphatidylethanolamine and N-(4-nitrobenzo-2-oxa-1,3-diazol)-phosphatidylethanolamine and by electron microscopy. The fusogenic activity was dependent on the presence of vesicular stomatitis virus glycoprotein, was pH-sensitive, and had a pH threshold of activation similar to that of the native virus. The extent of %%%fusion%%% was dependent upon the lipid composition of the vesicles. This technique will allow vesicles prepared by any method to be made fusogenic.

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12145757 BIOSIS NO.: 199497167042
Real-time measurements of chemically-induced membrane %%%fusion%%% in cell monolayers, using a %%%resonance%%% energy transfer method
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JOURNAL: Biochimica et Biophysica Acta 1189 (2): p175-180 1994 1994
ISSN: 0006-3002
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: %%%Fusion%%% of mouse melanoma cells grown in monolayers has been directly monitored by fluorescence %%%resonance%%% energy transfer between %%%fluorescein%%% and %%%rhodamine%%% probes attached to octadecanoic acid. Various poly(ethylene glycol)s (PEG), either alone or in combination with amphipathic molecules, have been used as fusogens. %%%Fusion%%% starts at a maximum rate as soon as PEG is removed from the medium and reaches a plateau after 20-30 min. Both the initial rate and extent of %%%fusion%%% have been recorded for each experiment. The extent of %%%fusion%%% shows in general a positive correlation with the initial rate, although PEGs with different molar masses appear to induce %%%fusion%%% at different rates, but to a similar extent. A good correlation has been found between the extent of %%%fusion%%%, as measured by fluorescence, and the '%%%fusion%%% index' computed from cell and nucleus counting: a calibration curve is provided for the interconversion of both parameters. Optimum %%%fusion%%% values are obtained with 50% (w/v) PEG 1500. The effect of pre-treatments with surfactants (Triton X-100, sodium dodecylsulphate) on PEG-induced %%%fusion%%% has also been tested. Sodium dodecylsulphate, but not Triton, enhances considerably both the rate and extent of cell %%%fusion%%%. The in situ generation of the amphipathic molecule diacylglycerol, through the catalytic activity of a phospholipase C, also enhances significantly the %%%fusion%%% parameters. These results are in

good agreement with previous studies based on syncitia counting.

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11979723 BIOSIS NO.: 199497001008
%%%Fusion%%% of dioleoylphosphatidylcholine vesicles induced by an amphiphilic cationic peptide and oligophosphates at neutral pH
AUTHOR: Murata Masayuki; Shirai Yoshinori; Ishiguro Ryo; Kagiwada Satoshi; Tahara Yoshikazu; Ohnishi Shun-Ichi; Takahashi Sho (Reprint)
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JOURNAL: Biochimica et Biophysica Acta 1152 (1): p99-108 1993 1993
ISSN: 0006-3002
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Peptide E5 is an analogue of the %%%fusion%%% peptide of influenza virus hemagglutinin and K5 is a cationic peptide which has an arrangement of electric charges complementary to that of E5. We reported that a stoichiometric mixture of E5 and K5 caused %%%fusion%%% of large unilamellar vesicles (LUV) of neutral phospholipids (Murata, M., Kagiwada, S., Takahashi, S. and Ohnishi, S. (1991) J. Biol. Chem. 266, 14353-14358). K5 caused %%%fusion%%% of LUV composed of dioleoylphosphatidylcholine (DOPC) at pH gt 10, but not at neutral pH. In the presence of oligophosphates, such as 1 mM ATP, GTP, or polyphosphate, K5 caused rapid and efficient %%%fusion%%% of DOPC LUV at neutral pH without hydrolysis of oligophosphate groups, but another anions such as citrate, acetate, AMP, phosphate, or EDTA were ineffective. The peptide/oligophosphate-induced %%%fusion%%% behaviors have been investigated by a fluorescence %%%resonance%%% energy transfer assay for lipid mixing of LUV and negative staining electron microscopy. At higher ionic strengths (gt 0.3 M KCl) or in the presence of 5.0 mM MgCl-2, the %%%fusion%%% was inhibited. Even at the inhibitory conditions, the association of K5 with lipid vesicles at neutral pH was directly confirmed by the Ficoll gradient assay method and by %%%blue%%% shifts of the tryptophan fluorescence of the peptide. A nonhydrolyzable GTP analogue, GTP-gamma-S, also induced %%%fusion%%%. These observations suggested that the electrostatic interactions between the positive and negative charges of K5 and oligophosphate, respectively, induced complex formation, triggering membrane %%%fusion%%%.

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11955466 BIOSIS NO.: 199396119882
Poly(ethylene glycol)-induced %%%fusion%%% and rupture of dipalmitoylphosphatidylcholine large, unilamellar extruded vesicles
AUTHOR: Massenburg Donald; Lentz Barry R (Reprint)
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JOURNAL: Biochemistry 32 (35): p9172-9180 1993
ISSN: 0006-2960

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RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: High concentrations (gtoreq 20 wt %) of poly(ethylene glycol) (PEG) induce large, unilamellar, dipalmitoylphosphatidylcholine model membrane vesicles to fuse when the bilayers contain small amounts of amphipathic peturbant molecules. In addition to %%%fusion%%%, similar concentrations of PEG induce these vesicles to leak their contents. In this paper, we have asked if %%%fusion%%% could occur independently of leakage or if %%%fusion%%% might be described as local bilayer rupture followed by resealing. By following the release of MW 10 000 %%%fluoresceinated%%% dextran trapped inside vesicles, it was determined that PEG-induced leakage was the result of major membrane disruption and not small-pore formation. %%%Fusion%%% of vesicles containing 0.5 mol % palmitic acid was clearly observed at 20 wt % PEG, while 25 wt % was needed to cause rupture. On the other hand, vesicles containing 0.5 mol % lysophosphatidylcholine ruptured at roughly the same concentration needed to induce rupture. Two methods were developed for removing PEG so that %%%fusion%%% products could be characterized. Quasi-elastic light scattering demonstrated that fusing vesicles grew in size and that nonfusing vesicles did not. Moreover, PEG concentrations that induced rupture led to the appearance of species with mean diameters much larger than those of fused vesicles. High-resolution nuclear magnetic %%%resonance%%% showed that the population of large vesicles that correlated with rupture was composed of multilamellar vesicles while the population resulting from %%%fusion%%% alone remained unilamellar. We conclude that, upon incubation with and subsequent removal of PEG, vesicles were either unaffected, or fused to form larger, unilamellar vesicles, or ruptured to form larger, nonunilamellar species.

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11838377 BIOSIS NO.: 199396002793
Motional dynamics of functional cytochrome c delivered by low pH %%%fusion%%% into the intermembrane space of intact mitochondria
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JOURNAL: Biochimica et Biophysica Acta 1142 (1): p194-202 1993
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LANGUAGE: English

ABSTRACT: We have investigated the motional dynamics of cytochrome c in the intact, functional rat liver mitochondrion. To do this, functional, FITC-cytochrome c (%%%fluorescein%%% isothiocyanate monoderivatized cytochrome c) was incorporated into the intermembrane space (IMS) of intact mitochondria through encapsulation of cytochrome c into asolectin liposomes followed by low pH-induced %%%fusion%%% of the liposomes with the outer membranes of the mitochondria. A cytochrome c controlled enrichment of between 15%-50% (1800-7200 molecules incorporated per mitochondrion) was obtained. All cytochrome c incorporated, regardless of

the quantity, participated in the function of electron transport, indicative of a functional, independent random diffusant. %%%Resonance%%% energy transfer was determined from the IMS-entrapped functional FITC-cytochrome c to octadecylrhodamine B incorporated into the mitochondrial membranes. %%%Resonance%%% energy transfer from FITC-cytochrome c to octadecylrhodamine B in isolated inner or outer mitochondrial membranes (IMM and OMM, respectively) was also measured. We found substantial differences in the effects of ionic strength (I) on the proximity of cytochrome c to isolated IMM and OMM. Interactions with isolated IMM were very dynamic, i.e., very I-dependent, and cytochrome c binding to IMM was significant only at very low I. I-dependent interactions of cytochrome c with isolated OMM were less I-dependent than those for the IMM. However, FITC-cytochrome c was essentially released from IMM and OMM at physiological I. The proximity of FITC-cytochrome c to each mitochondrial membrane after its incorporation into the IMS of intact mitochondria in the condensed configuration was estimated at different external, bulk I using: (a) %%%resonance%%% energy transfer from IMS-entrapped FITC-cytochrome c to octadecylrhodamine B-label evenly distributed in both mitochondrial membranes; and (b) %%%resonance%%% energy transfer from IMS-entrapped FITC-cytochrome c to octadecylrhodamine B-label concentrated in the OMM. %%%Resonance%%% energy transfer showed that the average distance between cytochrome c and the two IMS-membrane surfaces increased with increasing IMS-I, approaching a maximal measurable distance of 85 ANG at 150 mM I. This result is consistent with a dissociation of FITC-cytochrome c and both membranes of intact mitochondria at physiological I, i.e., when the activity of cytochrome c in electron transport is highest. Our findings reveal a primarily three-dimensional diffusion mode for IMS-cytochrome c during its function in electron transport in intact mitochondria at physiological I, and offer further evidence that mitochondrial electron transport is a process driven by random collisions between its independently diffusing electron transferring, redox components.

13/7/99
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11414367 BIOSIS NO.: 199294116208
PHOSPHOLIPID LIPOSOME %%%FUSION%%% BY THE FLUORESCENT PROBE METHOD
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JOURNAL: Journal of the Japan Oil Chemists' Society 41 (6): p501-506 1992
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LANGUAGE: JAPANESE

ABSTRACT: The %%%fusion%%% of naturally prepared liposomes and of liposome-biomembrane was investigated in terms of fluorescence %%%resonance%%% energy transfer using %%%fluorescein%%% (F) as a %%%resonance%%% energy donor and %%%rhodamine%%% B (R) as a %%%resonance%%% energy acceptor. Phospholipids were extracted from natural sources: phosphatidylcholine (PC) from egg yolk, phosphatidylserine (PS) from ox brain, and ghost %%%red%%% cell membrane (GRCM) from human blood. The %%%fusion%%% of small unilamellar vesicles

was induced by adding Ca2+ and then EDTA. The fluorescence probe method using liposomes labeled with probes was shown to be a simple way for monitoring liposome %%%fusion%%% between PS, PC and GRCM. In fluorescence spectra, the %%%fusion%%% of (F) labeled PS liposome PS (F) and (R) labeled PS liposome PS (R) showed a %%%rhodamine%%% peak due to %%%resonance%%% energy transfer higher than that of PC (F) and PC (R) liposomes. The %%%fusion%%% of PS liposomes is thus shown to occur much more easily than that of PC liposomes. PS liposomes fused with PC liposomes to form liposomes with higher PS content. Further study was made on the %%%fusion%%% of GRCM with PS liposomes to achieve a greater degree of %%%fusion%%% than of GRCM with PC liposomes.

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11379559 BIOSIS NO.: 199294081400
INTERACTION OF EOSINOPHIL GRANULE MAJOR BASIC PROTEIN WITH SYNTHETIC LIPID BILAYERS A MECHANISM FOR TOXICITY
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JOURNAL: Journal of Membrane Biology 128 (2): p153-164 1992
ISSN: 0022-2631
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RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: Eosinophil granule major basic protein (MBP) is a potent toxin for mammalian cells and helminths, but the mechanism of its toxicity is not known. Here we tested whether MBP toxicity is exerted through its effect on the lipid bilayer of its targets. Liposomes prepared from synthetic phospholipids were used as targets for MBP and their properties examined by fluorescence and circular dichroism (CD) spectroscopy. MBP caused a change in the temperature transition profiles of acidic liposomes (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphatidyl serine or an equimolar mixture of 1,2-dimyristoyl-sn-glycero-3-phosphocholine and 1,2-dimyristoyl-sn-glycero-3-phosphatidic acid) and induced their aggregation as shown by fluorescence %%%resonance%%% energy transfer experiments. The CD spectra and fluorescence characteristics of MBP itself were altered by its interaction with acidic lipids. %%%Blue%%% shifts in the emission maxima of the Trp, and of the dimethylaminonaphthyl moiety in acrylodan-labeled MBP, and a reduction in the effectiveness of quenching of Trp fluorescence by acrylamide were observed in the presence of acidic lipids. None of these effects were noted with zwitterionic lipids. This MBP:lipid bilayer interaction resulted in %%%fusion%%% and lysis of liposomes as indicated by the fluorescent indicator calcein. The results demonstrate that MBP associates with acidic lipids and that it disrupts, aggregates, fuses, and lyses liposomes prepared from such lipids. Such interaction might account for its wide range of toxicity.

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11176052 BIOSIS NO.: 199293018943
HEPATIC HEMANGIOMAS DIAGNOSIS WITH %%%FUSION%%% OF MR CT AND TECHNETIUM-99M-LABELED %%%RED%%% BLOOD CELL SPECT IMAGES
AUTHOR: BIRNBAUM B A (Reprint); NOZ M E; CHAPNICK J; SANGER J J; MEGIBOW A J; MAGUIRE G Q; WEINREB J C; KAMINER E M; KRAMER E L
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JOURNAL: Radiology 181 (2): p469-474 1991
ISSN: 0033-8419
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RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: A method of image analysis was developed for correlation of hemangiomas detected at computed tomography (CT) and/or magnetic %%%resonance%%% (MR) imaging with increased blood pool activity evident at single photon emission CT (SPECT) performed after labeling of %%%red%%% blood cells with technetium-99m. Image analysis was performed in 20 patients with 35 known hepatic hemangiomas. After section thickness and pixel sizes of the different studies were matched, intrinsic landmarks were chosen to identify anatomically corresponding locations. Regions of interest (ROIs) drawn on the CT and/or MR images were translated, rotated, and reprojected to match the areas of interest on the corresponding SPECT images by means of a two-dimensional polynomial-based warping algorithm. Analysis of ROIs on 30 SPECT-MR and 20 SPECT-CT pairs of registered images provided absolute confirmation that 34 suspected hemangiomas identified on SPECT images correlated exactly with lesions seen on CT and/or MR images. Accuracy of %%%fusion%%% was within an average of 1.5 pixels .+-. 0.8 (.+-. 1 standard deviation). The technique enabled diagnostic confirmation of hemangiomas as small as 1.0 cm and proved useful for evaluating lesions located adjacent to intrahepatic vessels.

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10838221 BIOSIS NO.: 199192083992
ANNEXIN-MEDIATED MEMBRANE %%%FUSION%%% OF HUMAN NEUTROPHIL PLASMA MEMBRANES AND PHOSPHOLIPID VESICLES
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JOURNAL: Biochimica et Biophysica Acta 1066 (2): p239-244 1991
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LANGUAGE: ENGLISH

ABSTRACT: Membrane %%%fusion%%% was studied using human neutrophil plasma membrane preparations and phospholipid vesicles approximately 0.5 .mu.m in diameter and composed of phosphatidylserine and phosphatidylethanolamine in a ratio of 1 to 3. Liposomes were labeled with N-(7-nitrobenzo-2-oxa-1,3-diazol-4-yl) (NBD) and lissamine %%%rhodamine%%% B derivatives of phospholipids. Apparent %%%fusion%%% was

detected as increase in fluorescence of the %%%resonance%%% energy transfer donor, NBD, after dilution of the probes into unlabeled membranes. 0.5 mM Ca2+ alone was sufficient to cause substantial %%%fusion%%% of liposomes with a plasma membrane preparation but not with other liposomes. Both annexin I and des(1-9) annexin I caused a substantial increase in the rate of %%%fusion%%% under these conditions while annexin V inhibited %%%fusion%%%. %%%Fusion%%% mediated by des(1-9) annexin I was observed at Ca2+ concentrations as low as approximately 5 .mu.M, suggesting that the truncated form of this protein may be active at physiologically low Ca2+ concentrations. Trypsin treated plasma membranes were incapable of %%%fusion%%% with liposomes, suggesting that plasma membrane proteins may mediate %%%fusion%%%. Liposomes did not fuse with whole cells at any Ca2+ concentration, indicating that the cytoplasmic side of the membrane is involved. These results suggest that annexin I and unidentified plasma membrane proteins may play a role in Ca2+-dependent degranulation of human neutrophils.

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10816340 BIOSIS NO.: 199192062111
DEVELOPMENT OF A STREAK-CAMERA-BASED TIME-RESOLVED MICROSCOPE FLUORIMETER AND ITS APPLICATION TO STUDIES OF MEMBRANE %%%FUSION%%% IN SINGLE CELLS
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JOURNAL: Biochemistry 30 (26): p6517-6527 1991
ISSN: 0006-2960
DOCUMENT TYPE: Article
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LANGUAGE: ENGLISH

ABSTRACT: A time-resolved microscope fluorimeter based on a synchroscan streak camera and a fast pulsed laser system has been developed to measure the fluorescence lifetime decay under the fluorescence microscope. This system allows one to measure the nanosecond fluorescence lifetimes of fluorophores in a small spot (0.8-6.3 .mu.m diameter) in single cultured cells under a fluorescence microscope, while the cells are being viewed under a high-power objective lens. A signal acquisition time between a second and a minute was usually sufficient to obtain fluorescence decay curves with good quality for 103-105 fluorophores localized in 1 .mu.m2 domain. A signal-to-noise ratio better than 30 was obtained for .apprxeq. 30 000 %%%fluorescein%%%-labeled band 3 molecules in a 2 .mu.m2 region in a single human erythrocyte ghost after signal accumulation for 30 s. The measured lifetimes for a variety of fluorescent probes attached to proteins in solution and lipids in liposomes showed a good agreement with those measured in a cuvette under standard conditions by time-correlated single photon counting. With the development of this instrument, microscope fluorimetry has become a practical, straightforward, quantitative technique for investigation of molecular processes in single cells in culture. Time-resolved microscope fluorimetry has been applied to observe %%%fusion%%% of liposomes in vitro and that of endosomes in single cells by monitoring %%%resonance%%% energy transfer. Inspection of individual liposomes and endosomes

revealed the extent of %%%fusion%%% for each vesicle. Since the use of time-resolved microscope fluorimetry eliminates the need for subcellular fractionation or the complex correction procedures in steady-state microfluorimetry, it greatly simplifies the assay for endosome %%%fusion%%% in vivo. The results showed that extensive %%%fusion%%% of sequentially formed endosomes takes place all over the cell matrix in cultured cells. This suggests that extensive %%%fusion%%% with incoming endosomes takes place in many endosomal compartments, possibly sorting organelles, or that the early endosomes fuse with the preexisting network of tubular cisternae of the endosomal compartment at many points in the network. It is concluded that time-resolved microscope fluorimetry is a powerful noninvasive technique for studies of in situ biochemistry and biophysics using cells and tissues.

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10814427 BIOSIS NO.: 199192060198
EFFECTS OF THE %%%FUSION%%% PEPTIDE FROM MEASLES VIRUS ON THE STRUCTURE OF N METHYLDIOLEOYLPHOSPHATIDYLETHANOLAMINE MEMBRANES AND THEIR %%%FUSION%%% WITH SENDAI VIRUS
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JOURNAL: Biochimica et Biophysica Acta 1065 (1): p49-53 1991
ISSN: 0006-3002
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: 31P nuclear magnetic %%%resonance%%% spectroscopy (31P-NMR) was used to study phospholipid organization in hydrated preparations of N-methyl dioleoylphosphatidylethanolamine and a '%%%fusion%%% peptide' with the sequence: FAGVVLAGAALGVAAAAQI, which corresponds to the amino terminus of the F1 subunit of the membrane %%%fusion%%% protein of measles virus. These amino acids are believed to mediate syncytia formation, host-cell penetration and hemolysis by infectious virus. The presence of the peptide at 0.5 mole percent significantly facilitated the formation of isotropic 31P resonances. The effects at 1 mole percent peptide were substantially enhanced over the effects observed at 0.5 mole percent, leading to a decrease in the onset temperature of the formation of the isotropic 31P-NMR resonances by about 30.degree. C. The formation of such isotropic 31P-NMR resonances has been previously associated with an increased rate of %%%fusion%%% of large unilamellar vesicles composed of N-methyl dioleoylphosphatidylethanolamine. Enhanced %%%fusion%%% of octadecyl %%%rhodamine%%%-labelled Sendai virus with N-methyl dioleoylphosphatidylethanolamine large unilamellar vesicles was observed when the '%%%fusion%%% peptide' was incorporated into the large unilamellar vesicles.

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09653507 BIOSIS NO.: 198987101398
USE OF LIPOPHILIC FLUORESCENT PROBES FOR THE ISOLATION OF HYBRID CELLS IN FLOW CYTOMETRY
AUTHOR: TERTOV V V (Reprint); SAYADYAN H S; KALANTAROV G F; MOLOTKOVSKY J G ; BERGELSON L D; OREKHOV A N
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JOURNAL: Journal of Immunological Methods 118 (1): p139-144 1989
ISSN: 0022-1759
DOCUMENT TYPE: Article
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LANGUAGE: ENGLISH

ABSTRACT: Flow cytometry was used for the isolation of hybrid cells immediately after %%%fusion%%%. Precursor cells were stained by two lipophilic fluorescent probes: perylenoyl-labeled triglyceride (perylenoyl-TG, green fluorescence, 520 nm) and rhodaminyl-labeled triglyceride (rhodaminyl-TG, %%%red%%% fluorescence, > 580 nm). Since the maximum emission of perylenoyl-TG coincides with the maximum absorbance of rhodaminyl-TG, the two fluorescent dyes form an effective donor-acceptor pair. Cells stained by perylenoyl-TG (0.25-1 .mu.g/ml) at the excitation wavelength of 457 nm displayed high intensity of fluorescence in the green region (520 nm), and low intensity of fluorescence in the %%%red%%% region (> 580 nm). Using the same conditions, cells that were stained by rhodaminyl-TG displayed a low intensity of fluorescence in both regions. When cells were simultaneously labeled by perylenoyl-TG and rhodaminyl-TG (used in a concentration ratio of 1:10, respectively) essentially total energy transfer was observed, and the cells exhibited a high intensity of %%%red%%% fluorescence. After the %%%fusion%%% of cells which had been separately stained by perylenoyl-TG and rhodaminyl-TG, the hybrid cells containing the two fluorescent probes had a high intensity of %%%red%%% fluorescence. %%%Resonance%%% exitation energy transfer between the two fluorescent dyes permits effective sorting of hybrid cells by flow cytofluorometry.

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09641475 BIOSIS NO.: 198987089366
MECHANISM OF PROTEIN-INDUCED MEMBRANE %%%FUSION%%% %%%FUSION%%% OF PHOSPHOLIPID VESICLES BY CLATHRIN ASSOCIATED WITH MEMBRANE BINDING AND CONFORMATIONAL CHANGES
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JOURNAL: Biochemistry 28 (3): p1422-1428 1989
ISSN: 0006-2960
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: The clathrin-induced %%%fusion%%% of liposome membranes, the membrane binding of clathrin, and the conformational states of clathrin were investigated over a wide pH range using large unilamellar and

multilamellar vesicles composed of phosphatidylserine (PS), phosphatidylcholine (PC), PS/PC (2:1), PS/PC (1:1), or PS/PC (1:2). The pH profiles of clathrin-induced %%%fusion%%% of all types of liposomes containing PS showed biphasic patterns. Their pH thresholds were found in the pH range of 5-6 and shifted to lower pH values with decrease in the PS content. Similar shifts were observed in the pH profiles of clathrin binding to these vesicles, but the pH profiles of binding were different from the biphasic %%%fusion%%% patterns. With PC vesicles, only small degrees of %%%fusion%%% and clathrin binding were observed at pH 2-4. The pH dependences of the conformation and hydrophobicity of clathrin were determined by measuring the extent of the %%%blue%%% shift of the fluorescence maximum of 1-anilinonaphthalene-8-sulfonate in the presence of the protein, the fluorescence intensity of N-(1-anilinonaphthyl-4)maleimide bound to the clathrin molecule, the %%%resonance%%% energy transfer from its tryptophan to anilinonaphthyl residues, the partitioning of the protein in Triton X-114 solution, and the hydrophobicity index of clathrin using cis-parinaric acid. These measurements indicated that conformational change and exposure of hydrophobic regions occur below pH 6 and suggested that clathrin may adopt different conformational states in the pH region where it induced membrane %%%fusion%%%. In addition, the extents of inactivation of clathrin-induced %%%fusion%%% by preincubation of the protein below and above pH 4 were different. Clathrin formed insoluble aggregates at pH 4-6, and soluble aggregates below pH 4, suggesting that two distinct %%%fusion%%%-active states exist at pH 2-6, which might be related to the biphasic %%%fusion%%% patterns. These results suggest that clathrin-induced %%%fusion%%% of liposome membranes involves both protein binding to the membranes and a conformational change of clathrin accompanied by the exposure of its hydrophobic domains. Clathrin binding may induce close apposition of the membranes, while the conformational change may induce insertion of the protein molecule into the membrane to perturb the lipid bilayer. This study provides clues for elucidation of the general mechanisms of pH-dependent membrane %%%fusion%%% induced by proteins.

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09245076 BIOSIS NO.: 198886084997
CHARACTERIZATION OF THE PH-INDUCED %%%FUSION%%% OF LIPOSOMES WITH THE PLASMA MEMBRANE OF RYE PROTOPLASTS
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JOURNAL: Biochemistry 27 (15): p5671-5677 1988
ISSN: 0006-2960
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: We present evidence that at acid pH, liposomes composed of soybean lipids fuse with the plasma membrane of protoplasts isolated from rye leaves (Secale cereale L. cv Puma). Using the %%%resonance%%% energy transfer assay (RET), we determined the rate and extent of liposome and protoplast plasma membrane lipid mixing. The fluorescent donor-acceptor

pair was N-(7-nitro-2,1,3-benzoxadiazol-4-yl)phosphatidylethanolamine (N-NBD-PE) and N-(lissamine %%%rhodamine%%% B sulfonyl)phosphatidylethanolamine (N-Rh-PE). %%%Fusion%%% was substantial below pH 5, and the half-time of lipid mixing was fast (t1/2 on the order of minutes) and pH, concentration, and temperature dependent. The extent of liposome and protoplast %%%fusion%%% from the total amount of liposomes associated with the protoplasts was also determined by the RET assay. Protoplasts were incubated with fluorescent-labeled liposomes (5 min at 30.degree. C) at different pH values and then washed twice by centrifugation. The fluorescence spectra of the protoplast suspension permitted determination of the ratio of N-NBD-PE emission at 530 nm to the N-Rh-PE emission at 590 nm, which is a measure of the degree of lipid mixing. Addition of 2% (v/v) Triton X-100 to these suspensions permitted determination of the total amount of N-NBD-PE associated with the protoplasts. The amount of liposomes associated (fused and unfused) with protoplasts at pH 3.9 was approximately 9 times greater than that at pH 5.6. Approximately 64% of the liposomes associated with protoplasts were fused with the protoplasts at pH 3.9, and only 9% at pH 5.6. The transfer of liposome contents to the protoplast interior was studied with a method based on the fluorescence enhancement of a solution of calcein, initially confined in the liposomes at self-quenching concentrations. The kinetics of calcein release were very similar to those of lipid mixing. Fluorescence microscopy showed that after %%%fusion%%% with liposomes containing calcein, the protoplasts exhibited a strong diffuse fluorescence in the interior. Further evidence that the enhancement in calcein fluorescence was not due to release of the dye in the aqueous media came from experiments employing protoplast pellets. After %%%fusion%%% of protoplasts with calcein-containing liposomes, the protoplasts were washed by two centrifugations. Fluorescence intensity measurements and fluorescence microscopy observations showed that protoplasts in the pellets retained a strong calcein fluorescence.

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09185902 BIOSIS NO.: 198886025823
SUPEROXIDE DISMUTASE AND CATALASE CONJUGATED TO POLYETHYLENE GLYCOL INCREASES ENDOTHELIAL ENZYME ACTIVITY AND OXIDANT RESISTANCE
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JOURNAL: Journal of Biological Chemistry 263 (14): p6884-6892 1988
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: Covalent conjugation of superoxide dismutase and catalase with polyethylene glycol (PEG) increases the circulatory half-lives of these enzymes from < 10 min to 40 h, reduces immunogenicity, and decreases sensitivity to proteolysis. Because PEG has surface active properties and can induce cell %%%fusion%%%, we hypothesized that PEG conjugation could enhance cell binding and association of normally membrane-impermeable enzymes. Incubation of cultured porcine aortic endothelial cells with

125I-PEG-catalase or 125I-PEG-superoxide dismutase produced a linear, concentration-dependent increase in cellular enzyme activity and radioactivity. Fluorescently labeled PEG-superoxide dismutase incubated with endothelial cells showed a vesicular localization. Mechanical injury to cell monolayers, which is known to stimulate endocytosis, further increased the uptake of fluorescent PEG-superoxide dismutase. Endothelial cell cultures incubated with PEG-superoxide dismutase and PEG-catalase for 24 h and then extensively washed were protected from the damaging effects of reactive oxygen species derived from exogenous %%%xanthine%%% oxidase as judged by two criteria: decreased release of intracellular 51Cr-labeled proteins and free radical-induced changes in membrane fluidity, measured by elctron paramagnetic %%%resonance%%% spectroscopy of endothelial membrane proteins covalently labeled with 4-maleimido-2,2,6,6 tetramethylpiperidinooxyl. Addition of PEG and PEG-conjugated enzymes perturbed the spin-label binding enviroment, indicative of producing an increase in plasma membrane fluidity. Thus, PEG conjugation to superoxide dismutase and catalase enhances cell association of these enzymes in a manner which increases cellular enzyme activities and provides prolonged protection from partially reduced oxygen species.

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09181458 BIOSIS NO.: 198886021379
INTERACTIONS OF THE LOW MOLECULAR WEIGHT GROUP OF SURFACTANT-ASSOCIATED PROTEINS SP 5-18 WITH PULMONARY SURFACTANT LIPIDS
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JOURNAL: Biochemistry 27 (8): p2689-2695 1988
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LANGUAGE: ENGLISH

ABSTRACT: The interaction of the low molecular weight group of surfactant-associated protein, SP 5--18, with the major phospholipids of pulmonary surfactant was studied by fluorescence measurements of liposomal permeability and %%%fusion%%%, morphological studies, and surface activity measurements. The ability of SP 5-18 to increase the permeability of large unilamellar lipid vesicles was enhanced by the presence of negatively charged phospholipid. The permeability of these vesicles increased as the protein concentration was raised and the pH was lowered. SP 5-18 also induced leakage from liposomes made both from a synthetic surfactant lipid mixture and from lipids separated from SP 5-18 during its purification from %%%canine%%% sources. When SP 5-18 was added to egg phosphatidylglycerol liposomes, the population of liposomes which became permeable leaked all encapsulated contents, while the remaining liposomes did not leak at all. The extent of leakage was higher in the presence of 3 mM calcium. SP 5-18 also induced lipid mixing between two populations of egg phosphatidylglycerol liposomes in the presence of 3 mM calcium, as monitored by %%%resonance%%% energy transfer between two different fluorescent lipid probes, N-(7-nitro-2,1,3-benzoxadiazol-4-yl)phosphatidylethanolamine and

N-(lissamine %%%rhodamine%%% B sulfonyl)phosphatidylethanolamine. Negative-staining electron microscopy showed that the addition of SP 5-18 and 3 mM calcium produced vesicles twice the size of control egg phosphatidylglycerol liposomes. In addition, surface balance measurements revealed that the adsorption of liposomal lipids to an air/water interface was enhanced by the presence of SP 5-18, negatively charged phospholipids, and 3 mM calcium. These observations suggest a similar lipid dependence for the interactions observed in the fluorescence and adsorption experiments.

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09085368 BIOSIS NO.: 198885054259
LOW PH INDUCED MEMBRANE %%%FUSION%%% OF LIPID VESICLES CONTAINING PROTON-SENSITIVE POLYMER
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JOURNAL: Biochemistry 26 (25): p8145-8150 1987
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ABSTRACT: For the purpose of cytoplasmic delivery of aqueous content in liposomes through endosomes, we synthesized a pH-sensitive polymer, cetylacetyl(imidazol-4-ylmethyl)polyethylenimine (CAIPEI), which generates polycations at acidic pH. CAIPEI in its aqueous phase caused aggregation of sonicated vesicles composed of phosphatidylserine (PS) and phosphatidylcholine (PC) (molar ratio 1:4) when the pH of the solution was lowered. The polymer also induced membrane intermixing as measured by %%%resonance%%% energy transfer between vesicles containing N-(7-nitro-2,1,3-benz[d]oxadiazol-4-yl)phosphatidylethanolamine and those containing N-%%%Rhodamine%%% phosphatidylethanolamine at pH 4-5, while the addition of CAIPEI caused neither aggregation of PC vesicles nor the intermixing of liposomal membranes between PC and PC/PS vesicles at any pH. The CAIPEI-induced membrane intermixing was dependent on the polymer/vesicle ratio rather than on the polymer concentration. Then the polymer was incorporated into the bilayers of PC vesicles. These CAIPEI vesicles also caused membrane intermixing with liposomes containing PS under acidic conditions. The reconstituted CAIPEI did not reduce the trapping efficiency of vesicles or increase their permeability to glucose even at low pH. The vesicles caused the low pH induced aggregation and membrane intermixing with other negatively charged liposomes containing phosphatidic acid or phosphatidylglycerol. These results suggest that the protonation of the polymer at acidic pH endows the CAIPEI vesicles with the activity to fuse with negatively charged liposomes.

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09085364 BIOSIS NO.: 198885054255

LIPID MIXING DURING MEMBRANE AGGREGATION AND %%%FUSION%%% WHY %%%FUSION%%% ASSAYS DISAGREE
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RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: The kinetics of lipid mixing during membrane aggregation and %%%fusion%%% was monitored by two assays employing %%%resonance%%% energy transfer between N-(7-nitro-2,1,3-benzoxadiazol-4-yl)phosphatidylethanolamine (NBD-PE) and N-(lissamine %%%Rhodamine%%% B sulfonyl)phosphatidylethanolamine (Rh-PE). For the "probe mixing" assay, NBD-PE and Rh-PE were incorporated into separate populations of phospholipid vesicles. For the "probe dilution" assay, both probes were incorporated into one population of vesicles, and the assay monitored the dilution of the molecules into the membrane of unlabled vesicles. The former assay was found to be very sensitive to aggregation, even when the internal aqueous contents of the vesicles did not intermix. Examples of thois case were large unilamellar vesicles (LUV) composed of phosphatidylserine (PS) in the presence of Mg2+ and small unilamellar vesicles (SUV) composed of phosphatidylserine in the presence of high concentrations of Na+. No lipid mixing was detected in these cases by the probe dilution assay. Under conditions where membrane %%%fusion%%% (defined as the intermixing of aqueous contents with concomitant membrane mixing) was observed, such as LUV (PS) in the presence of Ca2+, the rate of probe mixing was faster than that of probe dilution, which in turn was faster than the rate of content mixing. Two assays monitoring the intermixing of aqueous contents were also compared. The Tb/dipicolinic acid assay reported slower %%%fusion%%% rates than the 1-aminonaphthalene-3,6,8-trisulfonic acid/N,N'-p-xylylene-bis(pyridinium bromide) assay for PS LUV undergoing %%%fusion%%% in the presence of Ca2+. These observations point to the importance of utilizing contents mixing assays in conjunction with lipid mixing assays to obtain the rates of membrane destabilization and %%%fusion%%%. They also indicate that the results of probe mixing assays have to be interpreted cautiously and that such assays can be sensitive to simple aggregation.

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08729835 BIOSIS NO.: 198784083984
NOVEL FLUORESCENT PHOSPHOLIPIDS FOR ASSAYS OF LIPID MIXING BETWEEN MEMBRANES
AUTHOR: SILVIUS J R (Reprint); LEVENTIS R; BROWN P M; ZUCKERMANN M
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JOURNAL: Biochemistry 26 (14): p4279-4287 1987
ISSN: 0006-2960
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: A series of fluorescent phospholipids has been synthesized, by a general and versatile procedure, with various fluorescent groups attached to the methyl-terminal half of one acyl chain in an otherwise normal phospholipid structure. Phospholipids labeled with (dialkylamino)coumarin moieties, and to a slightly lesser extent those labeled with a bimane group, exhibit a strong and stable %%%blue%%% fluorescence in phospholipid dispersions that is relatively insensitive to the physical state of the lipid phase. The fluorescence of these labeled phospholipids is efficiently quenched by %%%resonance%%% energy transfer to lipids labeled with a [[(dimethylamino)phenyl]azo]phenyl or a methyl(nitrobenzoxadiazolyl)amino group when these acceptors are incorporated into the same bilayer as the donor species. Acyl chain labeled phospholipid probes, both of whose chains are at least sixteen carbons in length, exchange extremely slowly between lipid vesicles (< 1% exchange/h). These properties allow various donor-acceptor combinations of probes to be employed in sensitive and reliable assays of lipid mixing accompanying membrane %%%fusion%%%. We demonstrate tht, in two particularly demanding applications (assays of the calcium-mediated coalescence of phosphatidylserine vesicles and of the proton-triggered coalescence of phosphatidylethanolamine vesicles), some combinations of acyl chain labeled probes offer substantial advantages over the commonly used N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)phosphatidylethanolamine/N-(lissamine %%%rhodamine%%% B sulfonyl)phosphatidylethanolamine pair to monitor accurately the progress of lipid mixing between vesicles.

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08718343 BIOSIS NO.: 198784072492
ALUMINUM-INDUCED LIPID PHASE SEPARATION AND MEMBRANE %%%FUSION%%% DOES NOT REQUIRE THE PRESENCE OF NEGATIVELY CHARGED PHOSPHOLIPIDS
AUTHOR: DELEERS M (Reprint); SERVAIS J-P; WULFERT E
AUTHOR ADDRESS: CENT RECH, UCB SA, SECTEUR PHARMACEUTIQUE, CHEMIN FORIEST, B-1420 BRAINE-L'ALLEUD, BELGIUM**BELGIUM
JOURNAL: Biochemistry International 14 (6): p1023-1034 1987
ISSN: 0158-5231
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RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: The interaction of aluminum with phosphatidyl serine lipid vesicles containing variable amounts of phosphatidyl ethanolamine, phosphatidyl choline and cholesterol has been studied by lipid phase separation monitored by fluorescence quenching. The interaction of Al3+ with neutral phospholipid membranes has also been investigated. Maximal lipid phase separation can be demonstrated in mixed phosphatidyl ethanolamine - cholesterol vesicles when using concentrations of aluminum between 87.5 and 125 .mu.M. Millimolar concentrations of Ca2+, Mn2+, Cd2+ and Zn2+ were without any effect. Aluminum also induced %%%fusion%%% of phospholipid membranes monitored by %%%resonance%%% energy transfer between N-(7-nitro-2,1,3, benzoxadiazol-4 yl) phosphatidyl ethanolamine and N-(lissamine %%%Rhodamine%%% B-sulfonyl) phosphatidyl ethanolamine, either when containing low amounts of phosphatidyl serine (12.5%) or

without any negatively charged phospholipid. Aluminum-induced %%%fusion%%% of liposomes was also monitored by the fluorescence of the terbium-dipicolinic acid complex (Tb-DPA3-) formed during %%%fusion%%% of vesicles containing either Tb-(citrate)6- complex or sodium salt of dipicolinic acid.

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08698755 BIOSIS NO.: 198784052904
LOW PH %%%FUSION%%% OF MOUSE LIVER NUCLEI WITH LIPOSOMES BEARING COVALENTLY BOUND LYSOZYME
AUTHOR: ARVINTE T (Reprint); WAHL P; NICOLAU C
AUTHOR ADDRESS: DEP MED BIOCHEM GENETICS, TEXAS A AND M UNIV, COLL MED, COLLEGE STATION, TEX 77843-1112, USA**USA
JOURNAL: Biochimica et Biophysica Acta 899 (2): p143-150 1987
ISSN: 0006-3002
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RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: Lysozyme covalently bound to liposomes induces the %%%fusion%%% of liposomes with isolated mouse liver nuclei. The %%%fusion%%% behavior is very similar to the case of erythrocyte ghosts (Arvinte, T., Hildenbrand, K., Wahl, P. and Nicolau, C. (1986) Proc. Natl. Acad. Sci. USA 83, 962-966). Kinetic studies showed that membrane lipid mixing was complete within 15 min, as indicated from the %%%resonance%%% energy transfer (RET) measurements. For the %%%resonance%%% energy transfer kinetic measurements the liposomes contained L-.alpha.-dipalmitoylphosphatidylethanolamine (DPPE), labeled at the free amino group with the energy donor 7-nitrobenz-2-oxa,-1,3-diazol-4-yl (NBD) or with the energy acceptor tetramethylrhodamine. The lipid mixing at equilibrium was studied by the fluorescence recovery after photobleaching technique (FRAP). Liposomes (with/without lysozyme) containing Rh-labeled DPPE in their membranes were incubated with nuclei at 37.degree. C, pH 5.2, for 30 min. After washing of nuclei by three centrifugations, 60-70% of the initial amount of labeled DPPE was associated with the nuclei in the case of liposomes bearing lysozyme and only 7-10% in the case of liposomes without lysozyme. For the nuclei incubated with liposomes having lysoyzme, about 70% of the total Rh-labeled lipids present in the nuclei diffused in the nuclear membrane(s) (lateral diffusion constant of D = (1.4 .+-. 0.5) .cntdot. 10-9 cm2/s). By encapsulating %%%fluorescein%%% isothiocyanate-labeled dextran of 150 kDa molecular mass into the liposomes and using a microfluorimetric method, it was shown that after the %%%fusion%%% a part of the liposome contents is found in the nuclei interior. In this lysozyme-induced %%%fusion%%% process between liposomes and nuclei or erythrocyte ghosts, the binding of lysozyme to the glycoconjugates contained in the biomembranes at acidic pH seems to be the determining step which explains the high fusogenic property of the liposomes bearing lysozyme.

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08646574 BIOSIS NO.: 198784000723
FACILITATION OF ELECTROFUSION OF PLANT PROTOPLASTS BY MEMBRANE-ACTIVE AGENTS
AUTHOR: NEA L J (Reprint); BATES G W; GILMER P J
AUTHOR ADDRESS: DEP BIOL SCI, THE INST MOL BIPHYS, FLA STATE UNIV, TALLAHASSEE, FL 32306, USA**USA
JOURNAL: Biochimica et Biophysica Acta 897 (2): p293-301 1987
ISSN: 0006-3002
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: Protoplasts isolated from a suspension culture of Daucus carota were subjected to electrofusion by means of a combination of dielectrophoresis to align the cells and brief d.c. shocks to induce %%%fusion%%%. The protoplasts were treated with agents known to alter membrane structure and function in order to define the factors that limit electrofusion. Lysophosphatidylcholine, dimethylsulfoxide and calcium chloride enhanced electrofusion; low temperature reduced %%%fusion%%%. However, lysophosphatidylcholine, calcium chloride, and low temperature all decreased membrane fluidity as measured by electron spin %%%resonance%%% spectroscopy of intact carrot protoplasts labeled with 5-doxylstearic acid. These spectra were taken inthe presence of .**GRAPHIC**. and thus largely reflect the fluidity of the plasma membrane. Treating the protoplasts with pronase or proteinase K also facilitated electrofusion. The proteinase K effect was largely reversed by the specific protease inhibitor phenylmethylsulfonyl fluoride. Protoplasts labeled with %%%fluorescein%%% isothiocyanate-concanavalin A did not exhibit any perceptible capping or clustering of membrane proteins in response to protease treatment. Enhancement of protoplast electrofusion by proteases may be due to improvement of cell-cell contacts through the removal of membrane surface determinants. However, the possibility that proteases enhance electrofusion by causing aggregation of membrane proteins cannot be eliminated.

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08614746 BIOSIS NO.: 198783093637
%%%RESONANCE%%% ENERGY-TRANSFER AND FLUORESCENCE INTENSITY STUDIES OF THE TRANSPORT OF LIPOSOME-ENCAPSULATED MOLECULES INTO ISOLATED MOUSE LIVER NUCLEI
AUTHOR: ARVINTE T (Reprint); WAHL P; NICOLAU C
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JOURNAL: Biochemistry 26 (3): p765-772 1987
ISSN: 0006-2960
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: We present evidence that liposome (composed of egg yolk L-.alpha.-phosphatidylcholine/phosphatidylethanolamine/cholesterol, in a

molar ratio of 4:5:1) fuse with isolated mouse liver nuclei at low pH. Using the %%%resonance%%% energy-transfer assay, we determined the rate and extent of liposome and nuclear membrane lipid mixing. %%%Fusion%%% was substantial when the pH was below 5. The half-time of lipid mixing decreased by acidification of the solvent, reaching about 2 min at pH 4.5. In order to study the transport of the liposome-aqueous contents to the interior of the nuclei during the process, we developed fluorescence assays in which %%%fluorescein%%% isothiocyanate labeled dextrans of 150 kDa molecular mass (FITC-D150) were encapsulated in liposomes. These liposomes also included in their bilayers the fluoresecent lipid N-tetramethylrhodamine-L-.alpha.-dipalmitoylphosphatidylethanolamine (N-Rh-DPPE). After incubation of these liposomes with mouse liver nuclei (pH 4.5, 37.degree. C, 30 min), we measured the fluorescence spectra of a suspension of washed nuclei and of nuclei treated by the detergent Triton X-100 (membrane-denuded nuclei). These Triton X100 treated nuclei had no N-Rh-DPPE fluorescence while they showed a FItC-D150 fluorescence which amounted to 20% of that of the intact nuclei. In another assay, a laser beam was focused on single nuclei by a microscope epiexcitation device. The variation of the N-Rh-DPPE and FITC-D150 fluorescence with the nuclear radius was determined with the microphotometric attachment of the microscope. For the N-Rh-DPPE which was confined on the nuclear surface, the fluorescence intensity decrased strongly when the nucleus radius increased, as expected from the optical sectioning property of the microscope objective. This decrease was much weaker with the FITC-D150 fluorescence, showing that the dextran was partly distributed in the nuclear volume. By fitting these variations with formulas giving the light collection efficiency of the objective [Koppel, D. E., Axelrod, D., Schlessinger, J., Elson, E. L., and Webb, W. W. (1976) Biophys. J. 16, 1315-1329], we determined that 30% of the nucleus-associated dextrans were in the nucleus interior. To our knowledge, these are the first reported results showing that liposomes fuse with isolated mouse liver nuclei and that the liposome contents can be transferred into the nucleus interior. Our work suggests that liposome %%%fusion%%% with the nuclear membrane might be one of the steps in the process of transfer of liposome-encapsulated genes to eukaryote cells [Wong, T. K., Nicolau, C., and Hofschnieder, P. H. (1980) Gene 10, 87-94; Soriano, P., Dijkstra, J., Legrand, A., Spanjer, H., Lodos-Gagliardi, D., Roerdink, F., Scherphof, G., and Nicolau, C. (1983) Proc. Natl. Acad. Sci. U.S.A. 80, 7128-7131].

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08233113 BIOSIS NO.: 198682079500
ROLE OF MEMBRANE HYDRATION AND MEMBRANE FLUIDITY IN THE MECHANISM OF ANION-INDUCED %%%FUSION%%% OF DIDODECYLDIMETHYLAMMONIUM BROMIDE VESICLES
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JOURNAL: Journal of the American Chemical Society 108 (14): p3920-3925 1986
ISSN: 0002-7863
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: The mechanism by which dianions of dipicolinic acid (DPA2-) induce %%%fusion%%% of didodecyldimethylammonium bromide (DDAB) vesicles has been investigated. Labeling of the DDAB bilayers with fluorescent probe molecules indicates that the alterations in the hydrophilic head group region are essential for %%%fusion%%% to occur. An isothermal phase transition in the vesicle bilayer is not a prerequisite for %%%fusion%%%. The effect of binding DPA2- to the vesicles causes a %%%blue%%% shift in the emission spectrum of one of the membrane-associated probes and a concomitant increase in the fluorescence quantum yield. These results together with the increase in fluorescence polarization are interpreted in terms of dehydration of the bilayer-water interface by DPA2- and an increase in the lateral packing of the DDAB head groups. Since these effects already occur below the DPA2- aggregation threshold concentration, a subsequent process appears to be required to initiate the actual %%%fusion%%% event. The possible formation of a specific "trans" (interbilayer) dianion-DDAB complex is discussed. Monitoring the kinetics of %%%fusion%%% as a function of temperature with a membrane %%%fusion%%% assay based on %%%resonance%%% energy transfer shows that the vesicles have a low tendency to fuse below the phase transition temperature (17.degree. C) although a transient increase in the %%%fusion%%% rate is observed around the pretransition temperature (7.degree. C). DDAB vesicles appear to be most susceptible to %%%fusion%%% when the bilayers are in an overall fluid state.

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08204175 BIOSIS NO.: 198682050562
SYNERGISTIC EFFECTS OF MICROMOLAR CONCENTRATIONS OF ZINC AND CALCIUM ON MEMBRANE %%%FUSION%%%
AUTHOR: DELEERS M (Reprint); SERVAIS J-P; WULFERT E
AUTHOR ADDRESS: CENT RECH, UCB, SA-SECTEUR PHARMACEUTIQUE CHEMIN FORIEST, B-1420 BRAINE-L'ALLEUD, BELGIUM**BELGIUM
JOURNAL: Biochemical and Biophysical Research Communications 137 (1): p 101-107 1986
ISSN: 0006-291X
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: %%%Resonance%%% Energy Transfer between N-(7-nitro-2,1,3 benzoxadiazol-4 yl) phosphatidyl ethanolamine and N-Lissamine-%%%Rhodamine%%% B sulfonyl phosphatidyl ethanolamine embedded in two different populations of small unilamellar vesicles made of phosphatidyl serine has been used to study the %%%fusion%%% process induced by Zn2+ and Ca2+. Lipid intermixing demonstrating %%%fusion%%% of liposome membranes can already be observed at 125 and 250 .mu.mol/l of Zn2+. After short time pre-incubations with micromolar concentrations of Zn2+ as low as 150 .mu.mol/l, Ca2+ induces an instantaneous increase of vesicle %%%fusion%%%. The lipid intermixing induced by micromolar concentrations of Ca2+ (250-500 .mu.mol/l) could be increased up to 4 times when pre-incubated with 150 or 200 .mu.mol/l of Zn2+. The effect of 1 mM of Ca2+ alone on lipid intermixing can be mimicked by 150 .mu.mol/l of Zn2+ followed by 500 .mu.mol/l of Ca2+. Our data demonstrate that Zn2+ and Ca2+ act synergistically to affect cation-induced membrane %%%fusion%%%.

We suggest that Zn2+ specifically alters the physical state of phospholipid membranes making them more prone to calcium-triggered %%%fusion%%%.

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08184455 BIOSIS NO.: 198682030842
CALCIUM-PHOSPHATE-INDUCED IMMOBILIZATION OF FLUORESCENT PHOSPHATIDYLSERINE IN SYNTHETIC BILAYER MEMBRANES INHIBITION OF LIPID TRANSFER BETWEEN VESICLES
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JOURNAL: Biochemistry 25 (8): p2141-2148 1986
ISSN: 0006-2960
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: %%%Resonance%%% energy transfer between 4-nitro-2,1,3-benzoxadiazole (NBD) acyl chain labeled phospholipid analogues and (lissamine) %%%rhodamine%%% B labeled phosphatidylethanolamine was used to monitor the rate of NBD-labeled lipid transfer between a variety of small unilamellar donor vesicles and dioleoylphosphatidylcholine (DOPC) acceptor vesicles. In the presence of appropriate concentrations of Ca2+ and phosphate, the transfer rate of NBD-phosphatidylserine (NBD-PS) from vesicles composed of lipid extracts from human %%%red%%% blood cells was reduced by .apprx. 10-fold, while the transfer rates of NBD-phosphatidylcholine, -ethanolamine, -glycerol, -N-succinylethanolamine, and -phosphatidic acid were essentially unaffected. A systematic evaluation of the lipid composition needed to facilitate the Ca2+/phosphate-induced inhibition of NBD-PS transfer revealed that the process was dependent upon the inclusion of both cholesterol and phosphatidylethanolamine (PE) in the donor vesicle population. Inhibition of NBD-PS transfer required the sequential addition of phosphate and Ca2+ to the vesicles, indicating that the combined interaction of Ca2+ and phosphate at the membrane surface was a prerequisite for inhibition to occur. Parallel experiments designed to determine the possible mechanism of this phenomenon showed that inhibition of NBD-PS transfer was not due to Ca2+-mediated phasic separations or vesicle-vesicle %%%fusion%%%. However, the addition of Ca2+ and phosphate to vesicles composed of total %%%red%%% blood cell lipids or cholesterol/PE did result in their aggregation. On the other hand, aggregation per se did not seem to be responsible for the inhibition of transfer since NBD-PS-containing vesicles composed of DOPC or DOPC/DOPE also aggregated, although NBD-PS transfer was unaffected. Our data show that PS can be immobilized by Ca2+ and phosphate in model bilayer membranes containing cholesterol and PE. These results suggest that Ca2+ and phosphate might induce the formation of intramembrane complexes with PS. The potential implication of such a mechanism for the maintenance of PS asymmetry in biological membranes is discussed.

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08150023 BIOSIS NO.: 198681113914
NEUROTOXIC CATIONS INDUCED MEMBRANE RIGIDIFICATION AND MEMBRANE %%%FUSION%%% AT MICROMOLAR CONCENTRATIONS
AUTHOR: DELEERS M (Reprint); SERVAIS J-P; WULFERT E
AUTHOR ADDRESS: CENTRE RECH, UCB, SA, SECTEUR PHARM, CHEMIE FORIEST, B-1420 BRAINE-L'ALLEUD, BELG**BELGIUM
JOURNAL: Biochimica et Biophysica Acta 855 (2): p271-276 1986
ISSN: 0006-3002
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: The effect of the neurotoxic cations aluminum, cadmium and manganese on membranes was examined in sonicated unilamellar vesicles containing phosphatidylserine and compared to the effect of Ca2+. %%%Fusion%%% of membranes was monitored by assessing the %%%resonance%%% energy transfer between N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)phosphatidylethanolamine and N-(lissamine-%%%rhodamine%%% B-sulfonyl)phosphatidylethanolamine. Self-quenching of high concentrations of carboxyfluorescein in liposomes was used to demonstrate the release of molecules entrapped in liposomes to compare the kinetics of leakage and intermixing of lipid. Rigidification of membranes was evaluated by monitoring the fluorescence polarization of 1,6-diphenyl-1,3,5-hexatriene embedded in membranes containing phosphatidylserine and dipalmitoylphosphatidylcholine. Cation-induced lipid intermixing of vesicles membranes and release of dye from the vesicles occurred in the same concentration range. With aluminum, these effects were observed with concentrations less than 25 .mu.M. Significant rigidification of vesicle membranes was apparent with less than 25 .mu.M of Al3+. Similar effects could only be observed with concentrations of Cd2+ and Mn2+ at least one order of magnitude higher (200 and 400 .mu.M, respectively).

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07787492 BIOSIS NO.: 198580096387
PARAMETERS AFFECTING LOW-PH-MEDIATED %%%FUSION%%% OF LIPOSOMES WITH THE PLASMA MEMBRANE OF CELLS INFECTED WITH INFLUENZA VIRUS
AUTHOR: VAN MEER G (Reprint); DAVOUST J; SIMONS K
AUTHOR ADDRESS: EUROPEAN MOLECULAR BIOLOGY LABORATORY, D-6900 HEIDELBERG, FRG**WEST GERMANY
JOURNAL: Biochemistry 24 (14): p3593-3602 1985
ISSN: 0006-2960
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: Unilamellar liposomes can be fused at low pH with the plasma membrane of cells that express the hemagglutinin glycoprotein of influenza virus on their surface. This %%%fusion%%% process was resolved into 2 kinetically distinct steps. The first and more rapid step converts

the bound liposome to a form that can no longer be released by neuraminidase. The 2nd step is the actual membrane %%%fusion%%% as measured by the loss of %%%resonance%%% energy transfer between 2 liposomal fluorescent phospholipids, N-(7-nitro-2,1,3-benzoxadiazol-4-yl)dioleoylphosphatidylethanolamine (N-NBD-PE) and N-(lissamine %%%rhodamine%%% B sulfonyl)dioleoylphosphatidylethanolamine (N-Rh-PE). In contrast to the 1st step, the rate of the 2nd one was highly dependent on the liposomal lipid composition and the cell type used. The replacement of 50% of the phosphatidylcholine (PC) in egg PC-cholesterol liposomes by unsaturated phosphatidylethanolamine (PE) species increased the rate of %%%fusion%%% at least 2-fold. Of the PE-containing liposomes that were associated with Madin-Darby %%%canine%%% kidney (MDCK) cells after 30 s of %%%fusion%%%, 80% had actually fused with the plasma membrane. Fringe pattern fluorescence photobleaching experiments showed that after %%%fusion%%% a fraction of the cell-associated N-Rh-PE diffused laterally in the plasma membrane. Without %%%fusion%%%, the N-Rh-PE was completely immobile. Under optimal conditions, the mobile fractions were 65% on MDCK cells and 78% on baby hamster kidney cells. The mobility was acquired simultaneously with the dilution of the fluorescent phospholipids as measured from the loss of %%%resonance%%% energy transfer. The mobile fraction of N-Rh-PE on the cell surface can therefore be used as a 2nd independent measure of actual membrane %%%fusion%%%. Upon %%%fusion%%% up to 80% of the nonexchangeable liposome markers cholesterol [14C]oleate and glycerol tri[14C]oleate became accessible to cellular hydrolases. This hydrolysis assay can also be used to monitor the 2nd step of the %%%fusion%%% process.

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07719228 BIOSIS NO.: 198580028123
RAPID KINETICS OF CALCIUM-INDUCED %%%FUSION%%% OF PHOSPHATIDYLSERINE-PHOSPHATIDYLETHANOLAMINE VESICLES THE EFFECT OF BILAYER CURVATURE ON LEAKAGE
AUTHOR: MORRIS S J (Reprint); GIBSON C C; SMITH P D; GREIF P C; STIRK C W; BRADLEY D; HAYNES D H; BLUMENTHAL R
AUTHOR ADDRESS: NEUROTOXICOLOGY SECTION, LABORATORY OF EXPERIMENTAL NEUROPATHOLOGY, NATIONAL INSTITUTE OF NEUROLOGICAL COMMUNICATIVE DISORDERS AND STROKE, BETHESDA, MD 20205, USA**USA
JOURNAL: Journal of Biological Chemistry 260 (7): p4122-4127 1985
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: Both small unilamellar vesicles (SUV) and large unilamellar vesicles formed by the reverse phase evaporation technique (REV) were used to study the initial kinetics of membrane aggretation and %%%fusion%%%. Stopped flow measurements of the Ca-induced changes in the turbidity of SUV and REV, formed from 1:1 (mol/mol) mixtures of bovine phosphatidylserine (PS) and Escherichia coli phosphatidylethanolamine (PE), were used to follow particle aggregation. Simultaneous measurements of the fluorescence %%%resonance%%% energy transfer from N-(7-nitro-2,1,3-benzoxadiazol-4-yl) (NBD)-PE to %%%rhodamine%%% (Rho)-PE

incorporated into the vesicle bilayers established that both initial aggregation and %%%fusion%%% can be described as a bimolecular process and the rate-limiting step of membrane %%%fusion%%% is aggregation. Thus %%%fusion%%% takes place in the microsecond time domain. Parallel experiments, which simultaneously measured aggregation and the dequenching of encapsulated carboxyfluorescein (CF) in the presence and absence of antifluorescein antibodies in the suspension medium, established that the small unilamellar vesicles rapidly lose their contents of CF as they fuse. The first few cycles of %%%fusion%%% of the large unilamellar vesicles are nonleaky, but leakage develops within 1-2 s as the particles grow in size. Thus the SUV are poor models for the study of nonleaky %%%fusion%%%, while the REV must be carefully tested before unambiguous interpretation of %%%fusion%%% assays involving the formation of tight complexes (such as the terbium-dipicolinic assay) can be made. NBD-PE undergoes very rapid, Ca2+-promoted changes in quantum yield which can obscure the %%%resonance%%% energy transfer signals. Thus data from the NBD-PE/Rho-PE energy transfer pair must be carefully scrutinized for artifacts.

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07611161 BIOSIS NO.: 198579030060
CALCIUM-PROMOTED %%%FUSION%%% OF ISOLATED CHROMAFFIN GRANULES DETECTED BY %%%RESONANCE%%% ENERGY TRANSFER BETWEEN LABELED LIPIDS EMBEDDED IN THE MEMBRANE BILAYER
AUTHOR: MORRIS S J (Reprint); BRADLEY D
AUTHOR ADDRESS: NEUROTOXICOLOGY SECT, LAB EXP NEUROPATHOLOGY, NATL INST NEUROLOGICAL COMMUNICATIVE DISRODERS STROKE, NATL INST HEALTH, BETHESDA, MD 20205, USA**USA
JOURNAL: Biochemistry 23 (20): p4642-4650 1984
ISSN: 0006-2960
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: Chromaffin granules isolated from bovine adrenal medulla were labeled with small unilamellar vesicles (SUV) made from N-(7-nitro-2,1,3-benzoxadiazol-4-yl)phosphatidylethanolamine and/or N-(lissamine %%%rhodamine%%% B sulfonyl)phosphatidylethanolamine as donor and acceptor fluorophores. Labeling of granules could be followed by the relief of self-quenching experienced when the labels were incorporated into the granule membrane. The mechanism of incorporation seemed to be %%%fusion%%% of the SUV with the granule membrane although transfer of label could not be ruled out. Granule-granule %%%fusion%%% could be detected either by %%%resonance%%% energy transfer from donor to acceptor fluorophores or by donor quenching in either of 2 different experimental designs: by a decrease in donor fluorescence and an increase in acceptor fluorescence when donor-labeled granules fused with acceptor-labeled granules or by an increase in donor fluorescence, and a decrease in acceptor fluorescence when granules containing both probes fused with unlabeled granules. %%%Fusion%%% of freshly prepared granules could be initiated by millimolar concentrations of Ca; Mg was less effective. Mg-ATP had no effect. %%%Fusion%%% was inhibited by potassium glutamate and a variety of organic and inorganic cations and anions, which also

inhibited granule-granule aggregation to a lesser extent. The conditions for promotion and inhibition of granule-granule %%%fusion%%% were quite different from those reported by Knight and Baker for exocytosis of granule contents from permeabilized chromaffin cells. The membrane %%%fusion%%% seen here is probably activated by a different mechanism.

13/7/124
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06843349 BIOSIS NO.: 198375027292
DYNAMICS AND TOPOGRAPHICAL DISTRIBUTION OF SURFACE GLYCO PROTEINS DURING MYO BLAST %%%FUSION%%% A %%%RESONANCE%%% ENERGY TRANSFER STUDY
AUTHOR: HERMAN B A (Reprint); FERNANDEZ S M
AUTHOR ADDRESS: PHYSIOL DEP, UNIV CONNECTICUT HEALTH CENT, FARMINGTON, CONNECTICUT 06032, USA**USA
JOURNAL: Biochemistry 21 (14): p3275-3283 1982
ISSN: 0006-2960
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: Changes in topograhy and lateral translational mobility of concanavalin A (Con A) receptors on the surface of cultured chick muscle cells were investigated during the period of myoblast %%%fusion%%%. A temporal correlation between these phenomena and the alteration in membrane fluidity known to occur during this time period were established. Receptor topograhy and mobility were studied by means of a %%%resonance%%% energy transfer technique employing pyrene- and FITC[%%%fluorescein%%%-isothiocyanate]-Con A conjugates. All measurements were performed through a microscope on single cells. During the period of myoblast %%%fusion%%%, Con A receptors underwent a dramatic redistribution on the cell surface. The changes in membrane fluidity observed during muscle differentiation served to modulate the lateral mobility of these receptors.

13/7/125
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06496600 BIOSIS NO.: 198273000527
USE OF %%%RESONANCE%%% ENERGY TRANSFER TO MONITOR MEMBRANE %%%FUSION%%%
AUTHOR: STRUCK D K (Reprint); HOEKSTRA D; PAGANO R E
AUTHOR ADDRESS: DEP EMBRYOL, CARNEGIE INSTITUTION WASHINGTON, BALTIMORE, MARYLAND 21210, USA**USA
JOURNAL: Biochemistry 20 (14): p4093-4099 1981
ISSN: 0006-2960
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: An assay for vesicle-vesicle %%%fusion%%% involving %%%resonance%%% energy transfer between N-(7-nitro-2,1,3-benzoxadiazol-4-yl), the energy donor, and %%%rhodamine%%%, the energy acceptor, was developed. The 2 fluorophores

are coupled to the free amino group of phosphatidylethanolamine to provide analogs which can be incorporated into a lipid vesicle bilayer. When both fluorescent lipids are in phosphatidylserine vesicles at appropriate surface densities (ratio of fluorescent lipid to total lipid), efficient energy transfer is observed. When such vesicles are fused with a population of pure phosphatidylserine vesicles by the addition of Ca, the 2 probes mix with the other lipids present to form a new membrane. This mixing reduces the surface density of the energy acceptor resulting in a decreased efficiency of %%%resonance%%% energy transfer which is measured experimentally. These changes in transfer efficiency allow kinetic and quantitative measurements of the %%%fusion%%% process. Using this system, the ability of phosphatidylcholine, phosphatidylserine and phosphatidylcholine-phosphatidylserine (1:1) vesicles to fuse with cultured fibroblasts [Chinese hamster V79] was studied. Under the conditions used, the majority of the cellular uptake of vesicle lipid could be attributed to the adsorption of intact vesicles to the cell surface regardless of the composition of the vesicle bilayer.

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06354611 BIOSIS NO.: 198274086058
ELEMENTARY RECONSTITUTION OF THE WATER SPLITTING LIGHT REACTION IN PHOTOSYNTHESIS 2. OPTICAL DOUBLE %%%RESONANCE%%% STUDY OF HYDRATED CHLOROPHYLL A 2 PHOTON INTERACTIONS IN NONPOLAR SOLUTIONS
AUTHOR: ALFANO A J (Reprint); FONG F K
AUTHOR ADDRESS: DEP CHEM, PURDUE UNIV, WEST LAFAYETTE, INDIANA 47907, USA** USA
JOURNAL: Journal of the American Chemical Society 104 (10): p2767-2773 1982
ISSN: 0002-7863
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: Two-photon interactions of (Chl a.cntdot.2H2O)n [hydrated chlorophyll from spinach] in deaerated 1:1 n-pentane-methylcyclohexane solutions were investigated by fluorescence quenching measurements obtained by using time-resolved optical methods. The Chl a triplet state was populated by excitation with a modulated laser beam. The decay of the triplet population was monitored by measuring the quenching of Chl a fluorescence excited by a CW [continuous wave] laser beam. The reliability of instrumental response in the dual-excitation fluorescence experiments is ensured by electronic simulations by using a %%%red%%% -light emitting diode simultaneously driven by a 52-Hz waveform and a weaker DC input. Flux dependence measurements were analyzed in terms of the steady-state population of the first excited singlet, S1, under illumination by both lasers and of the flux density of the modulated laser beam. Experimental criteria were given for identifying the biphotonic mechanism responsible for the observed effects. The rationale for establishing singlet-triplet %%%fusion%%% as the mechanism underlying the observed behavior is provided. The possible significance of the 2-photon mechanism in the Chl a water splitting reaction relative to the stereospecific interactions in hydrated Chl a aggregation is discussed.

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19229954 BIOSIS NO.: 200600575349
AMY-1 (associate of Myc-1) localization to the trans-Golgi network through interacting with BIG2, a guanine-nucleotide exchange factor for ADP-ribosylation factors
AUTHOR: Ishizaki Ray; Shin Hye-Won; Iguchi-Ariga Sanae M M; Ariga Hiroyoshi (Reprint); Nakayama Kazuhisa
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JOURNAL: Genes to Cells 11 (8): p949-959 AUG 2006 2006
ISSN: 1356-9597
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: AMY-1 (associate of Myc-1) was originally identified as a c-Myc-binding protein that enhances the c-Myc transcription activity, and subsequently found to interact with A-kinase-anchoring proteins (AKAPs), including AKAP149, S-%%%AKAP84%%% and AKAP95. We show here that, using anti-AMY-1 antibodies we raised, AMY-1 localizes to the trans-Golgi network (TGN) and the nucleus. To explore the possible function of AMY-1, we have undertaken a search for interacting partners by co-immunoprecipitation experiments using cells stably expressing FLAG-tagged AMY-1. Interestingly, we have found that AMY-1 interacts with BIG2 and BIG1, both of which are high molecular weight guanine-nucleotide exchange factors for ADP-ribosylation factors (ARFs) and mainly localize to the TGN. Furthermore, we have demonstrated that AMY-1 is associated with the TGN through interacting with BIG2 but not with BIG1 using an RNA interference approach, although AMY-1 can interact with both BIG1 and BIG2 in vitro. Taken together with the facts that BIG2 contains domains that bind to regulatory subunits of protein kinase A and that recruitment of ARF1 onto Golgi membranes is mediated, at least in part, by activation of protein kinase A, these results suggest that BIG2 alone or in concert with recruited AMY-1 coordinates ARF-mediated membrane trafficking and signaling pathways.

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17136658 BIOSIS NO.: 200300095377
AMY-1 interacts with S-%%%AKAP84%%% and AKAP95 in the cytoplasm and the nucleus, respectively, and inhibits cAMP-dependent protein kinase activity by preventing binding of its catalytic subunit to A-kinase-anchoring protein (AKAP) complex.
AUTHOR: Furusawa Makoto; Taira Takahiro; Iguchi-Ariga Sanae M M; Ariga Hiroyoshi (Reprint)

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JOURNAL: Journal of Biological Chemistry 277 (52): p50885-50892 December 27, 2002 2002
MEDIUM: print
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: We have reported that a novel c-Myc-binding protein, AMY-1, binds to cAMP-dependent protein kinase-anchoring protein 149 (AKAP149) and its splicing variant, %%%AKAP84%%% and is localized in the mitochondria in a complex with RII, a regulatory subunit of cAMP-dependent protein kinase (PKA) (Furusawa, M., Ohnishi, T., Taira, T., Iguchi-Ariga, S. M. M., and Ariga, H. (2001) J. Biol. Chem. 276, 36647-36651). In this study, we further found that AMY-1 competitively bound to either AKAP95 or %%%AKAP84%%% in the nucleus and the cytoplasm, respectively, in a concentration-dependent manner of either AKAP. Like %%%AKAP84%%%, AMY-1 was found to bind to the RII-binding region of AKAP95 in vivo and in vitro and to make a ternary complex with RII. It was also found that the formation of the complex of AMY-1 with %%%AKAP84%%%/95 and RII prevented a catalytic subunit from binding to this AKAP complex, leading to suppression of PKA activity. These findings suggest that AMY-1 is an important modulator of PKA.

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17096974 BIOSIS NO.: 200300055693
AAT-1, a novel testis-specific AMY-1-binding protein, forms a quaternary complex with AMY-1, A-kinase anchor protein 84, and a regulatory subunit of cAMP-dependent protein kinase and is phosphorylated by its kinase.
AUTHOR: Yukitake Hiroshi; Furusawa Makoto; Taira Takahiro; Iguchi-Ariga Sanae M M; Ariga Hiroyoshi (Reprint)
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JOURNAL: Journal of Biological Chemistry 277 (47): p45480-45492 November 22, 2002 2002
MEDIUM: print
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: AMY-1 has been identified by us as a c-Myc-binding protein and was found to stimulate c-Myc transcription activity. AMY-1 was also found to be associated with protein kinase A anchor protein 84/149 (S-%%%AKAP84%%%/AKAP149) in the mitochondria in somatic cells and sperm, suggesting that it plays a role in spermatogenesis. To determine the molecular function of AMY-1, a two-hybrid screening of cDNAs encoding AMY-1-binding proteins was carried out with AMY-1 as a bait using a human testis cDNA library, and a clone encoding a novel protein, AAT-1, was

obtained. Three isoforms of AAT-1, AAT-1alpha, -beta, and -gamma, were found to be derived from an alternative splicing of the transcripts of the aat-1 gene, which was mapped at human chromosome 3q13-3q21. AAT-1 was found to be specifically expressed in the testis during the course of spermatogenesis and also to be present in the spermatid and mature sperm, as was AMY-1. AAT-1alpha was found to bind to and be colocalized in mitochondria with AMY-1 in human HeLa and mouse GC-1 cells. Furthermore, AAT-1alpha was found to bind to the N-terminal half of S-%%%AKAP84%%% /AKAP149 in a quaternary complex with AMY-1 and a regulatory subunit (RII) of cAMP-dependent kinase (PKA), in which AAT-1alpha was associated with RII via S-%%%AKAP84%%%/AKAP149, in rat testis and HeLa cells. It was then found that AAT-1alpha weakly stimulated a phosphorylation activity of PKA and also that AAT-1 itself was phosphorylated by PKA in vivo and in vitro. These results suggest that both AAT-1 and AMY-1 play roles in spermatogenesis.

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16918869 BIOSIS NO.: 200200512380
AMAP-1, a novel testis-specific AMY-1-binding protein, is differentially expressed during the course of spermatogenesis
AUTHOR: Yukitake Hiroshi; Furusawa Makoto; Taira Takahiro; Iguchi-Ariga Sanae M M; Ariga Hiroyoshi (Reprint)
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JOURNAL: Biochimica et Biophysica Acta 1577 (1): p126-132 19 August, 2002
2002
MEDIUM: print
ISSN: 0006-3002
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: AMY-1 has been identified by us as a c-Myc-binding protein and was found to stimulate c-Myc transcription activity. AMY-1 was also found to be associated with %%%AKAP84%%%/149 in the mitochondria in somatic cells and sperm, suggesting that it plays a role in spermatogenesis. To access the molecular function of AMY-1, a two-hybrid screening of cDNAs encoding AMY-1-binding proteins was carried out with AMY-1 as a bait using a human testis cDNA library, and a clone encoding a novel protein, AMAP-1, was obtained. The amap-1 gene was mapped at human chromosome 17q21. AMY-1 was found to bind to and be colocalized with AMAP-1 in human 293T and HeLa cells. AMAP-1 was found to be specifically expressed in the testis and expressed post-meiotically in the testis, as was AMY-1. These results suggest that both AMAP-1 and AMY-1 play roles in spermatogenesis.

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16865646 BIOSIS NO.: 200200459157
A-kinase anchor protein 84/121 are targeted to mitochondria and mitotic

spindles by overlapping amino-terminal motifs
AUTHOR: Cardone Luca; de Cristofaro Tiziana; Affaitati Adelina; Garbi Corrado; Ginsberg Michael D; Saviano Michele; Varrone Stelio; Rubin Charles S; Gottesman Max E; Avvedimento Enrico V; Feliciello Antonio (Reprint)
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JOURNAL: Journal of Molecular Biology 320 (3): p663-675 12 July, 2002 2002
MEDIUM: print
ISSN: 0022-2836
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: A-kinase anchor proteins (AKAPs) assemble multi-enzyme signaling complexes in proximity to substrate/effector proteins, thus directing and amplifying membrane-generated signals. S-%%%AKAP84%%% and AKAP121 are alternative splicing products with identical NH2 termini. These AKAPs bind and target protein kinase A (PKA) to the outer mitochondrial membrane. Tubulin was identified as a binding partner of S-%%%AKAP84%%% in a yeast two-hybrid screen. Immunoprecipitation and co-sedimentation experiments in rat testis extracts confirmed the interaction between microtubules and S-%%%AKAP84%%%. In situ immunostaining of testicular germ cells (GC2) shows that AKAP121 concentrates on mitochondria in interphase and on mitotic spindles during M phase. Purified tubulin binds directly to S-%%%AKAP84%%% but not to a deletion mutant lacking the mitochondrial targeting domain (MT) at residues 1-30. The MT is predicted to form a highly hydrophobic alpha-helical wheel that might also mediate interaction with tubulin. Disruption of the wheel by site-directed mutagenesis abolished tubulin binding and reduced mitochondrial attachment of an MT-GFP fusion protein. Some MT mutants retain tubulin binding but do not localize to mitochondria. Thus, the tubulin-binding motif lies within the mitochondrial attachment motif. Our findings indicate that S-%%%AKAP84%%%/AKAP121 use overlapping targeting motifs to localize signaling enzymes to mitochondrial and cytoskeletal compartments.

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16545849 BIOSIS NO.: 200200139360
Characterization of RIIbeta and D-AKAP1 in differentiated adipocytes
AUTHOR: Chaudhry Archana (Reprint); Zhang Chen; Granneman James G
AUTHOR ADDRESS: Pfizer Global Research and Development, 2800 Plymouth Road, Ann Arbor, MI, 48105, USA**USA
JOURNAL: American Journal of Physiology 282 (1 Part 1): pC205-C212 January, 2002 2002
MEDIUM: print
ISSN: 0002-9513
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: A-kinase anchoring proteins (AKAPs) have been proposed to regulate cAMP-dependent signaling in the cell by targeting RII subunits of protein kinase A (PKA) to specific subcellular compartments. RIIbeta is the predominant PKA subtype in adipose tissue. In gel overlay assays of C3H/10T1/2 adipocytes and adipose tissue, RIIbeta bound to several proteins including a prominent 132-kDa band, which was strongly induced upon differentiation of C3H/10T1/2 cells into adipocytes. Immunoblotting and nuclease protection analysis of C3H/10T1/2 cellular extracts identified this band as D-AKAP1/S-%%%AKAP84%%%, a putative AKAP. Immunocytochemical analysis of C3H/10T1/2 adipocytes revealed that most of D-AKAP1/S-%%%AKAP84%%%, but not RIIbeta, was colocalized with a mitochondrial-selective dye, MitoTracker red. These findings were further confirmed in studies where D-AKAP1/S-%%%AKAP84%%%, but not RIIbeta, were localized in purified mitochondria made from C3H/10T1/2 adipocytes. Moreover, D-AKAP1, which is upregulated after differentiation, did not recruit RIIbeta to membrane fractions enriched in mitochondria. These results demonstrate that D-AKAP1/S-%%%AKAP84%%% does not interact with PKA in differentiated C3H/10T1/2 adipocytes under the conditions tested.

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16331904 BIOSIS NO.: 200100503743
AMY-1, a c-Myc-binding protein, is localized in the mitochondria of sperm by association with S-%%%AKAP84%%%, an anchor protein of cAMP-dependent protein kinase
AUTHOR: Furusawa Makoto; Ohnishi Takako; Taira Takahiro; Iguchi-Ariga Sanae M M; Ariga Hiroyoshi (Reprint)
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JOURNAL: Journal of Biological Chemistry 276 (39): p36647-36651 September 28, 2001 2001
MEDIUM: print
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: We have reported that a novel c-Myc-binding protein, AMY-1 (associate of Myc-1), stimulated the transcription activity of c-Myc. To access the molecular function of AMY-1, a two-hybrid screening of cDNAs encoding AMY-1-binding proteins was carried out with AMY-1 as a bait using a human HeLa cDNA library, and a clone encoding cAMP-dependent protein kinase anchor protein 149 (AKAP149), was obtained. AMY-1 was found to bind in vitro and in vivo to the regulatory subunit II binding region of AKAP149 and S-%%%AKAP84%%%, a splicing variant of AKAP149 expressed in the testis. AMY-1 was expressed postmeiotically in the testis, as S-%%%AKAP84%%% was expressed. Furthermore, S-%%%AKAP84%%% and regulatory subunit II, a regulatory subunit of cAMP-dependent protein kinase, made a ternary complex in cells, and AMY-1 was localized in the mitochondria of HeLa and sperm in association with AKAP149 and S-%%%AKAP84%%%, respectively. These results suggest that AMY-1 plays a role in spermatogenesis.

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15627383 BIOSIS NO.: 200000345696
Analysis of A-kinase anchoring protein (AKAP) interaction with protein kinase A (PKA) regulatory subunits: PKA isoform specificity in AKAP binding
AUTHOR: Herberg Friedrich W (Reprint); Maleszka Ariane; Eide Turid; Vossebein Lutz; Tasken Kjetil
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JOURNAL: Journal of Molecular Biology 298 (2): p329-339 April 28, 2000 2000
MEDIUM: print
ISSN: 0022-2836
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Compartmentalization of cAMP-dependent protein kinase (PKA) is in part mediated by specialized protein motifs in the dimerization domain of the regulatory (R)-subunits of PKA that participate in protein-protein interactions with an amphipathic helix region in A-kinase anchoring proteins (AKAPs). In order to develop a molecular understanding of the subcellular distribution and specific functions of PKA isozymes mediated by association with AKAPs, it is of importance to determine the apparent binding constants of the R-subunit-AKAP interactions. Here, we present a novel approach using surface plasmon resonance (SPR) to examine directly the association and dissociation of AKAPs with all four R-subunit isoforms immobilized on a modified cAMP surface with a high level of accuracy. We show that both AKAP79 and S-%%%AKAP84%%%/D-AKAP1 bind RIIalpha very well (apparent KD values of 0.5 and 2 nM, respectively). Both proteins also bind RIIbeta quite well, but with three- to fourfold lower affinities than those observed versus RIIalpha. However, only S-%%%AKAP84%%%/D-AKAP1 interacts with RIalpha at a nanomolar affinity (apparent KD of 185 nM). In comparison, AKAP95 binds RIIalpha (apparent KD of 5.9 nM) with a tenfold higher affinity than RIIbeta and has no detectable binding to RIalpha. Surface competition assays with increasing concentrations of a competitor peptide covering amino acid residues 493 to 515 of the thyroid anchoring protein Ht31, demonstrated that Ht31, but not a proline-substituted peptide, Ht31-P, competed binding of RIIalpha and RIIbeta to all the AKAPs examined (EC50-values from 6 to 360 nM). Furthermore, RIalpha interaction with S-%%%AKAP84%%%/D-AKAP1 was competed (EC50 355 nM) with the same peptide. Here we report for the first time an approach to determine apparent rate- and equilibria binding constants for the interaction of all PKA isoforms with any AKAP as well as a novel approach for characterizing peptide competitors that disrupt PKA-AKAP anchoring.

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15616348 BIOSIS NO.: 200000334661

Localization of a novel human A-kinase-anchoring protein, hAKAP220, during spermatogenesis
AUTHOR: Reinton Nils; Collas Philippe; Haugen Trine B; Skalhegg Bjorn S; Hansson Vidar; Jahnsen Tore; Tasken Kjetil (Reprint)
AUTHOR ADDRESS: Institute of Medical Biochemistry, University of Oslo, Blindern, N-0317, Oslo, Norway**Norway
JOURNAL: Developmental Biology 223 (1): p194-204 July 1, 2000 2000
MEDIUM: print
ISSN: 0012-1606
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Using a combination of protein kinase A type II overlay screening, rapid amplification of cDNA ends, and database searches, a contig of 9923 bp was assembled and characterized in which the open reading frame encoded a 1901-amino-acid A-kinase-anchoring protein (AKAP) with an apparent SDS-PAGE mobility of 220 kDa, named human AKAP220 (hAKAP220). The hAKAP220 amino acid sequence revealed high similarity to rat AKAP220 in the 1167 C-terminal residues, but contained 727 residues in the N-terminus not present in the reported rat AKAP220 sequence. The hAKAP220 mRNA was expressed at high levels in human testis and in isolated human pachytene spermatocytes and round spermatids. The hAKAP220 protein was present in human male germ cells and mature sperm. Immunofluorescent labeling with specific antibodies indicated that hAKAP220 was localized in the cytoplasm of premeiotic pachytene spermatocytes and in the centrosome of developing postmeiotic germ cells, while a midpiece/centrosome localization was found in elongating spermatocytes and mature sperm. The hAKAP220 protein together with a fraction of PKA types I and II and protein phosphatase I was resistant to detergent extraction of sperm tails, suggesting an association with cytoskeletal structures. In contrast, S-%%%AKAP84%%%/D-AKAP1, which is also present in the midpiece, was extracted under the same conditions. Anti-hAKAP220 antisera coimmunoprecipitated both type I and type II regulatory subunits of PKA in human testis lysates, indicating that hAKAP220 interacts with both classes of R subunits, either through separate or through a common binding motif(s).

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15126922 BIOSIS NO.: 199900386582
Characterization of three genes, %%%AKAP84%%%, BAW and WSB1, located 3' to the neurofibromatosis type 1 locus in Fugu rubripes
AUTHOR: Kehrer-Sawatzki H (Reprint); Maier C; Moschgath E; Elgar G; Krone W
AUTHOR ADDRESS: Department of Human Genetics, University of Ulm, Albert-Einstein-Allee 11, 89081, Ulm, Germany**Germany
JOURNAL: Gene (Amsterdam) 235 (1-2): p1-11 July 22, 1999 1999
MEDIUM: print
ISSN: 0378-1119
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Sequence analysis of cosmid clones was instrumental to identify

three genes in the region flanking the Fugu rubripes NF1 gene in the 3' direction: the %%%AKAP84%%% gene (A-kinase anchor protein 84), the WSB1 gene (WD-40-repeat protein with a SOCS box) and the BAW gene of yet unknown function located between the %%%AKAP84%%% and the WSB1 genes. The human homologues of these genes are not located in the immediate vicinity of the NF1 gene at 17q11.2. Although synteny of the NF1, %%%AKAP84%%%, BAW and WSB1 genes is conserved between Fugu and human, the gene order is not conserved, and more than a simple inversion would have been necessary to explain the difference in gene order. The mammalian homologue of the Fugu BAW gene or protein has not yet been characterized. As deduced from the respective cDNAs, the Fugu %%%AKAP84%%%, WSB1 and BAW proteins vary concerning the overall degree of similarity to their mammalian counterparts. Whereas the overall similarity of %%%AKAP84%%% between Fugu and mouse is low, three regions of known functional importance show considerable conservation. These are the N-terminal anchoring domain mediating the insertion of %%%AKAP84%%% in the outer mitochondrial membrane, the binding site of the regulatory subunit (RI or RII) of protein kinase A, and the C-terminal domain present in the alternatively spliced isoform AKAP121 with an hnRNP K homology domain involved in RNA binding. A higher overall similarity of deduced protein sequences between Fugu and mouse was observed comparing the BAW gene products (74.1%) and the WSB1 proteins (77.2%).

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13975065 BIOSIS NO.: 199799609125
Organelle-specific targeting of protein kinase AII (PKAII): Molecular and in situ characterization of murine A kinase anchor proteins that recruit regulatory subunits of PKAII to the cytoplasmic surface of mitochondria
AUTHOR: Chen Qian; Lin Reigh-Yi; Rubin Charles S (Reprint)
AUTHOR ADDRESS: Dep. Mol. Pharmacol. F-229, Albert Einstein Coll. Med., 1300 Morris Park Ave., Bronx, NY 10461, USA**USA
JOURNAL: Journal of Biological Chemistry 272 (24): p15247-15257 1997 1997
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Experiments were designed to test the idea that A kinase anchor proteins (AKAPs) tether regulatory subunits (RII) of protein kinase AU (PKAII) isoforms to surfaces of organelles that are bounded by phospholipid bilayers. S-%%%AKAP84%%%, one of three RII-binding proteins encoded by a single-copy murine gene, was studied as a prototypic organelle-associated AKAP. When S-%%%AKAP84%%% was expressed in HEK293 cells, the anchor protein was targeted to mitochondria and excluded from other cell compartments. The RII tethering site is located in the cytoplasm adjacent to the mitochondrial surface. Endogenous RII subunits are not associated with mitochondria isolated from control cells. Expression of S-%%%AKAP84%%% in transfected HEK293 cells triggered a redistribution of 15% of total RII to mitochondria. Thus, the tethering region of the organelle-inserted anchor protein is properly oriented and avidly binds RII (PKAII) isoforms in intact cells. Two critical domains in S-%%%AKAP84%%% were mapped. Residues 1 to 30 govern insertion of the polypeptide into the outer mitochondrial membrane; amino acids 306-25

constitute the RII-binding site. Properties established for S-%%%AKAP84%%% in vitro and in situ strongly suggest that a physiological function of this protein is to concentrate and immobilize RII (PKAII) isoforms at the cytoplasmic face of a phospholipid bilayer.

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13867694 BIOSIS NO.: 199799501754
Identification of a novel protein kinase A anchoring protein that binds both type I and type II regulatory subunits
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JOURNAL: Journal of Biological Chemistry 272 (12): p8057-8064 1997 1997
ISSN: 0021-9258
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ABSTRACT: Compartmentalization of cAMP-dependent protein kinase is achieved in part by interaction with A-kinase anchoring proteins (AKAPs). All of the anchoring proteins identified previously target the kinase by tethering the type II regulatory subunit. Here we report the cloning and characterization of a novel anchoring protein, D-AKAP1, that interacts with the N terminus of both type I and type II regulatory subunits. A novel cDNA encoding a 125-amino acid fragment of D-AKAP1 was isolated from a two-hybrid screen and shown to interact specifically with the type I regulatory subunit. Although a single message of 3.8 kilobase pairs was detected for D-AKAP1 in all embryonic stages and in most adult tissues, cDNA cloning revealed the possibility of at least four splice variants. All four isoforms contain a core of 526 amino acids, which includes the R binding fragment, and may be expressed in a tissue-specific manner. This core sequence was homologous to S-%%%AKAP84%%%, including a mitochondrial signal sequence near the amino terminus (Lin, R. Y., Moss, S. B., and Rubin, C. S. (1995) J. Biol. Chem. 270, 27804-2781 1). D-AKAP1 and the type I regulatory subunit appeared to have overlapping expression patterns in muscle and olfactory epithelium by in situ hybridization. These results raise a novel possibility that the type I regulatory subunit may be anchored via anchoring proteins.

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13545547 BIOSIS NO.: 199699179607
Molecular characterization of AKAP149, a novel A kinase anchor protein with a KH domain
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JOURNAL: Biochemical and Biophysical Research Communications 225 (1): p

313-319 1996 1996
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RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The cytosolic cAMP activates in eukaryotic cells several isoforms of cAMP-dependent protein kinase (PKAs) involved in signal transduction. The effects of individual PKA isoforms are determined by their cellular localization, specified through binding to distinct A Kinase Anchor Proteins (AKAPs). A new member of the AKAP family, a membrane-anchored 903 amino acid long protein, designated AKAP149, is characterized in the present work. It is a putative splicing variant of S-%%%AKAP84%%% with the important new feature of a RNA-binding motif (KH domain). This domain together with the known characteristics of AKAPs suggests the involvement of AKAP149 in the phosphorylation-dependent regulation of RNA-processing.

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13133277 BIOSIS NO.: 199698601110
Characterization of S-%%%AKAP84%%%, a novel developmentally regulated a kinase anchor protein of male germ cells
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JOURNAL: Journal of Biological Chemistry 270 (46): p27804-27811 1995 1995
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ABSTRACT: In mammalian spermatozoa, most of the type II-alpha isoform of cAMP-dependent protein kinase (PKAII-alpha) is anchored at the cytoplasmic surface of a specialized array of mitochondria in the flagellar cytoskeleton. This places the catalytic subunits of PKAII-alpha in proximity with potential target substrates in the cytoskeleton. The mechanism by which PKAII-alpha is anchored at the outer surface of germ cell mitochondria has not been elucidated. We now report the cloning of a cDNA that encodes a novel, germ cell A kinase anchor protein (AKAP) designated S-%%%AKAP84%%%. S-%%%AKAP84%%% comprises 593 amino acids and contains a centrally located domain that avidly binds regulatory subunits (RII-alpha and RII-beta) of PKAII-alpha and PKAII-beta. The 3.2-kilobase S-%%%AKAP84%%% mRNA and the cognate S-%%%AKAP84%%% RII binding protein are expressed principally in the male germ cell lineage. Expression of S-%%%AKAP84%%% is tightly regulated during development. The protein accumulates as spermatids undergo nuclear condensation and tail elongation. The timing of S-%%%AKAP84%%% expression is correlated with the de novo accumulation of RII-alpha and RII-beta subunits and the migration of mitochondria from the cytoplasm (round spermatids) to the cytoskeleton (midpiece in elongating spermatids). Residues 1-30 at the NH-2 terminus of S-%%%AKAP84%%% constitute a putative signal/anchor sequence that may target the protein to the outer mitochondrial membrane. Immunofluorescence analysis demonstrated that S-%%%AKAP84%%% is co-localized with mitochondria in the flagellum.

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